THE NAUTILUS

Volume 134, Number 2
August 4, 2020
ISSN 0028-1344

A quarterly devoted to malacology.

SUBSCRIPTION INFORMATION

The subscription rate for volume 134 (2020) is US $65.00 for individuals, US $102.00 for institutions. Postage outside the United States is an additional US $10.00 for regular mail and US $28.00 for air delivery. All orders should be accompanied by payment and sent to: THE NAUTILUS, P.O. Box 1580, Sanibel, FL 33957, USA, (239) 395-2233.

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THE NAUTILUS (ISSN 0028-1344) is published quarterly by The Bailey-Matthews National Shell Museum, 3075 Sanibel-Captiva Road, Sanibel, FL 33957.

Periodicals postage paid at Sanibel, FL, and additional mailing offices.

POSTMASTER: Send address changes to: THE NAUTILUS
P.O. Box 1580
Sanibel, FL 33957

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CONTENTS

RESEARCH NOTES

Architectonica karsteni Rutsch, 1934 (Gastropoda: Architectonicidae) in seamounts of the Nazca-Desventuradas Marine Park: First record in Chilean waters since the Miocene

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ABSTRACT

Eight species of Architectonicidae have been reported for lower Miocene deposits in continental Chile. One of these species is *Architectonica karsteni*, which had an inferred geographic range, for this epoch, extending from Costa Rica to central Chile (~8° N to 34° S), and even in the Caribbean. There is no evidence of the current presence of the family immediately off the coast of Chile. We report the discovery of living specimens and shells of *A. karsteni* at four seamounts at ~200 m water depth in the recently created Nazca–Desventuradas Marine Park, located ~900 km west of Chile. Morphological identification was based on protoconch diameter, coloration patterns, and teleoconch sculpture. We also provide sequence data for portions of the mitochondrial COI and 16S rRNA genes as a contribution toward future population-level and phylogenetic analyses of this poorly known group. Insight on the habitat of the species based on underwater imagery is also provided. This new record extends the geographic distribution of *A. karsteni* ~20° southward from its current recorded range (i.e., Baja California to Peru). The finding of this species contributes to the knowledge of the fauna of these seamounts, and ultimately informs and boosts conservation efforts of these relatively pristine habitats.

Additional Keywords: Benthos, mesophotic zone, biogeography, Southeast Pacific Ocean, sundial shell, COI, 16S rRNA

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INTRODUCTION

Architectonicidae (commonly called "sundials") are a family of subtropical to tropical marine gastropods (Bieler, 1984). Their shell shape typically ranges from trochoidal to discoidal with a basal-centered umbilicus, but it may on occasion be planispiral with disjunct whorls. They are characterized by a hyperstrophic protoconch with the apex projected into the teleoconch umbilicus (Bieler, 1993). Architectonicids have planktotrophic larvae with larval durations that range from several weeks to several months (Robertson, 1967). Long duration of larval life allows the larvae to be displaced by currents over large distances (sometimes 1000s of km), therefore enabling them to maintain the wide geographical distributions reported for some species of this family (Bieler and Petit, 2005). For instance, several species, such as *Architectonica perspectiva* (Linnaeus, 1758) and *Heliacus variegatus* (Gmelin, 1791), have extremely wide geographic ranges, extending from the east coast of Africa (Indian Ocean) to the islands of the Central Pacific (Bieler, 1993). A few of them, including *Heliacus trochoides* (Gmelin, 1791) and *Psilaxis radiatus* (Röding, 1798), are known to extend to the eastern Pacific coast from the Indo-West and Central Pacific (Robertson, 1976, 1979). The distribution of architectonicid species may be limited by the temperature requirements of their cnidarian prey more than by their own tolerance (Bieler, 1993), since architectonicid larvae have been collected at water temperatures as low as 13.5 °C (Scheltema, 1971), and adults collected alive in deep waters at temperatures as low as 2.4 °C (Bieler, 1993). Changes in oceanographic

conditions, current patterns, and/or availability of prey may therefore have caused many species to have suffered reductions in their range of geographical distribution and to become extinct at high latitudes during the Miocene (Frassinetti and Covacevich, 1981; Nielsen and Frassinetti, 2007). For example, *Pseudotorinia obtusa* (Bronn, 1831) is known as a Neogene fossil from the North Sea (Janse and Janssen, 1983), has no extant representatives in that ocean (Melone and Taviani, 1984).

Such extinctions and contractions in geographic ranges have resulted in only two extant species of Architectonicidae having a known range that extends into Chilean waters. However, these species, *Heliacus implexus* (Mighels, 1845) and *Spirolaxis cornuarietis* (Bieler, 1993), are only known from Rapa Nui (Easter Island), which is ~3700 km west of continental Chile (Rehder, 1980; Bieler, 1993; Osorio, 2018). Nevertheless, samples collected from the Navidad Formation (~34° S, Figure 1), Ranquil Formation (~36.6° S) and Lemo Island (~44.64° S) reveal that at least eight species of the family were present in Chile during the lower Miocene (Frassinetti and Covacevich, 1981; Nielsen and Frassinetti, 2007). These species include: *Architectonica karsteni, Intitectonica inti* Frassinetti and Covacevich, 1982, *Discotectonica navidensis* Frassinetti and Covacevich, 1982, *Heliacus* (*Torinista*) *taverai* Frassinetti and Covacevich, 1981, *Heliacus* (*Torinista*) *bahamondei* Frassinetti and Covacevich, 1981, *Heliacus* (*Torinista*) *chonos* Nielsen and Frassinetti, 2007, and *Solatisonax bieleri* Nielsen and Frassinetti, 2007.

Architectonica karsteni was described by Rutsch (1934) from the Miocene Cantaure Fomation of northern Venezuela as a subspecies of *Architectonica nobilis* Röding, 1798. It was first reported for Chile as part of the fossil record of the Miocene by Frassinetti and Covacevich (1981). Additional records revealed that *A. karsteni* was consistently present in the lower Miocene fossil record in Central Chile and fossil records from the Miocene and Miocene-Pliocene boundary in Mexico (~16° N) (Böse, 1906), Venezuela and Grenadine Islands (DeVries, 1985; Nielsen and Frassinetti, 2007) (Figure 1). These records suggest that the geographic range of *A. karsteni* during the Miocene spanned the east Pacific and even the Caribbean Sea coasts, a wider longitudinal distribution relative to its current distribution. The present range of the species extends from Baja California (~32° N) to Perú (~5° S) (Figure 1) at 50 to 190 m depth on a variety of substrata (e.g., mud, fine sand, shells, gravel) (DeVries, 1985; Bieler, 1993). No records of living *A. karsteni* have been reported so far for the Pacific Ocean south of 5°S, the Caribbean Sea or the Atlantic (Bieler, 1993).

In this paper, we report the presence of *A. karsteni* at four seamounts near the junction of the Salas y Gómez and Nazca Ridges (Figure 1) (~25° S, 82° W) within the newly created Nazca-Desventuradas Marine Park (NDMP), Chile. In addition, we provide sequence data for the barcode regions of the mitochondrial (mt) 16S ribosomal RNA (rrnaL) and cytochrome oxidase I (COI) genes of *A. karsteni* and present them in a phylogenetic context with the only four Architectonicidae species with COI or 16S barcode data: *Architectonica perspectiva* (COI, 16S), *A. maxima* (16S), *Philippia lutea* (COI), and *Psilaxis radiatus* (16S). A description of the habitat of the species based on underwater imagery is also provided.

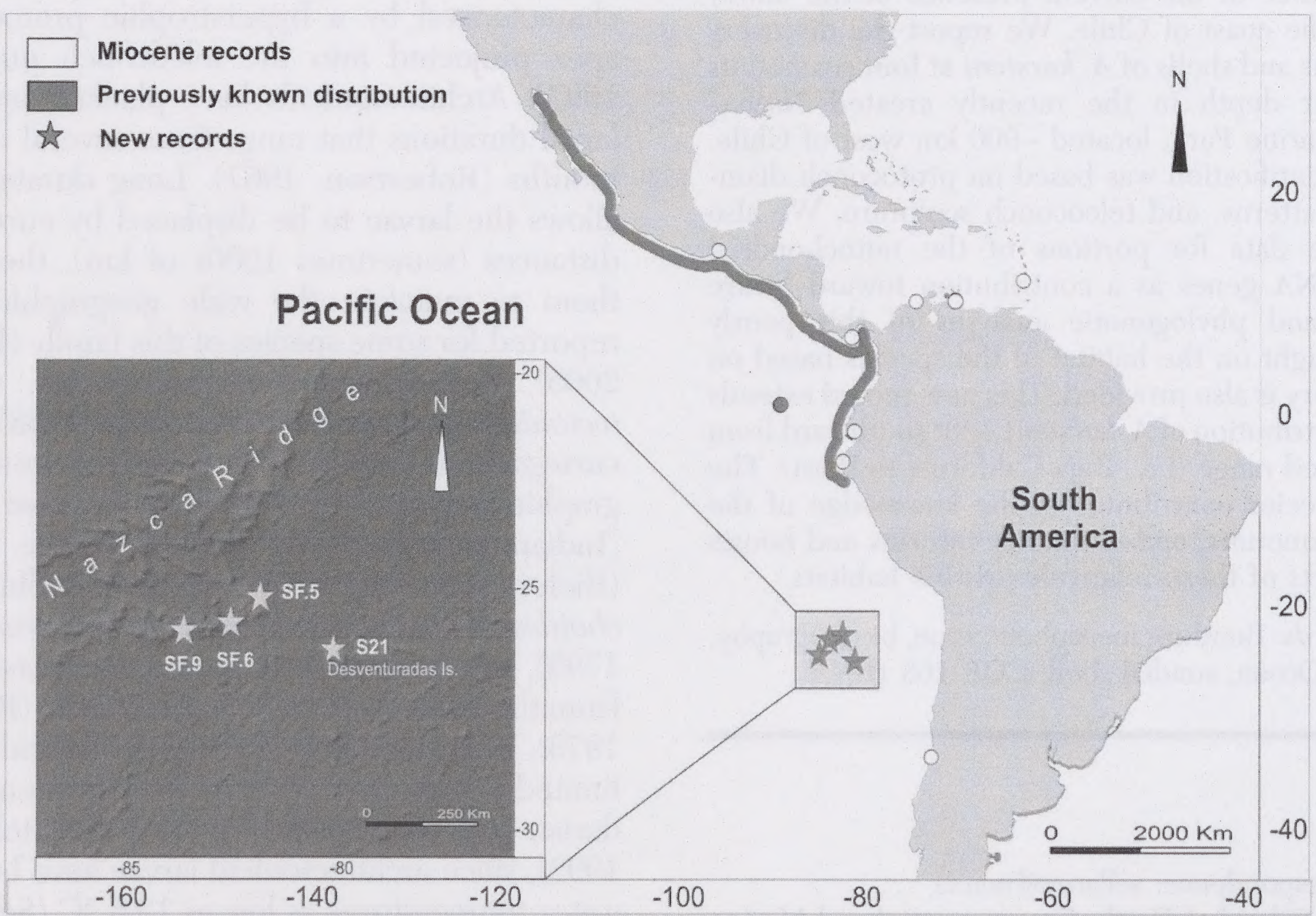


Figure 1. Modern and Miocene geographic distribution of *Architectonica karsteni* and locations of the new records reported here. Live specimens were only collected from seamount SF.5.

MATERIALS AND METHODS

Study Area: From October to November 2016, a multidisciplinary oceanographic cruise (CIMAR 22 "Oceanic Islands") was carried out onboard the research vessel AGS61 CABO DE HORNOS. The aim of the cruise was to study the benthic habitats and fauna of unexplored seamounts of the Juan Fernández and Desventuradas Ecoregion (Spalding et al., 2007). The northern extent of this ecoregion falls within the lower Miocene latitudinal range of *Architectonica karsteni* and includes the islands of the Desventuradas and the surrounding seamounts, which are part of the 144 seamounts of the Nazca and Salas y Gómez Ridges. The Salas y Gómez Ridge is a long and narrow seamount chain stretching from the East Pacific Rise (west of Easter Island in the Easter Island Ecoregion) at ~27°01′ S, 111°00′ W to just west of the Desventuradas Islands at ~25° 27 ′S, 81°43′ W, where it merges with the Nazca Ridge. This latter stretches in a northeastern direction to ~17°43′ S, 78°07′ W (Parin et al., 1997; Galvez Larach, 2009). Of these seamounts, 21 are in the Chilean exclusive economic zone (EEZ) around the Desventuradas (Yáñez et al., 2009). Prior to this study, benthic habitats of the seamounts within the EEZ surrounding the Desventuradas had not been explored. Some of the adjacent seamounts at the junction of these two ridges were surveyed in the 1970s and 1980s (Parin et al., 1997; Mironov et al., 2006). Such surveys revealed that these seamounts are characterized by a highly endemic fauna with well-expressed Indo-West Pacific affinities, with only the easternmost seamounts having some species with affinities to the fauna of the adjacent continental coast of South America (Parin, 1991).

Samples Collection: Within the NDMP, the slopes of San Ambrosio and San Felix islands (i.e., Desventuradas Islands) and summits of six seamounts were surveyed using a remotely operated vehicle (ROV) and a modified Agassiz trawl. Unless weather or sea conditions precluded the use of one of the survey methods, the protocol for the benthic survey consisted of a visual survey of the study site using an ROV (Commander MK2; Mariscope Meerestechnik, Kiel, Germany) equipped with a HD Camcorder (Panasonic SD 909) and laser pointers (10 cm apart) followed by sampling with the Agassiz trawl. The latter consisted of a metal frame with an opening of 1.5 m × 0.5 m (width × height) fitted with a net of 12 mm mesh at the cod end, operated in 10 min hauls (bottom contact) at ~2 knots. Collected specimens were preserved in 100% ethanol and voucher specimens deposited in the biological collections of the Universidad Católica del Norte (SCBUCN). Sample collection was performed under permission Res. Ext N°41/2016 from SERNAPESCA (Chile) to Universidad Católica del Norte. This resolution authorizes us to collect species throughout the NDMP area until October 2020.

Taxonomic Identification: Identification of the seven empty shells and three living specimens of architectonicids (Table 1) was made according to Bieler (1993). The teleoconch sculpture and protoconch diameter of one living specimen (ID number SCBUCN 6928a) was examined with a Hitachi SU3500 scanning electron microscope (SEM) at the Microscopy Laboratory of the Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile. The shell was dried in a laboratory drying oven at 65°C for 24 h and mounted on bronze stubs without metal coating.

Molecular Data: Whole genomic DNA was isolated from the muscle tissue of the foot of a specimen (ID number SCBUCN 6950) with the GeneJET Genomic DNA Purification Kit (ThermoFisher Scientific Waltham, MA) per manufacture's protocol and submitted to Biopolymers Facility at Harvard Medical School for library preparation and next-generation sequencing (NextSeq 500). Trimmed reads (Trimmomatic-0.32, Bolger et al., 2014) were assembled de novo with SPAdes (Bankevich et al., 2012) on the University of New Hampshire Bioinformatics Core facility RON server. The resulting SPAdes contig consensus sequences were blasted (Blastn) against the reference mitochondrial genomes in GenBank to identify which of the contigs corresponded to the mitochondrial genome of *A. karsteni*. After circularizing and editing overlapping ends of the identified SPAdes contig in Geneious R11.1.5 (Kearse et al., 2012), trimmed reads (BBDuk v. 37.25) were mapped to this reference sequence in Geneious and reviewed manually for quality

Table 1. Locations and depth of the seamounts sampled; and identification and measurements (diameter and height) of the *Architectonica karsteni* shells.

Station	Latitude	Longitude	Depth (m)	ID number SCBUCN	Diameter (mm)	Height (mm)	Sample alive
SF.5	–25.4272°	–81.8806°	180	6928a	28.4	15.4	Yes
				6950	22.3	11.2	No
				6926	21.2	10.2	No
				SF.5.029*	26.8	14.7	No
				SF.5.027*	28.4	14.0	Yes
				6928b	25.3	15.3	Yes
				6921	24.9	13.7	No
SF.6	–25.5535°	–82.3963°	176	7066	31.4	17.2	No
SF.9	–25.7774°	–83.3163°	200	6913	14.3	6.4	No
S21	–26.3790°	–79.8893°	150	7135	31.9	18.0	No

* Samples that do not have SCBUCN numbers, only field-assigned IDs

control (e.g., to confirm read coverage was sufficient, ≥10). The resulting consensus sequence was used to annotate the mitochondrial genome based on annotations identified by MITOS (Bernt et al., 2013) with gene boundaries manually adjusted based on alignment of individual genes with the gastropod *Siphonaria gigas* reference mitochondrial genome (NC_016188).

The complete COI and 16S rRNA sequences were uploaded to GenBank (MN270389 and MN270388, respectively) and aligned separately with default MUSCLE (Edgar, 2004) parameters to all Architectonicidae sequences available in GenBank. Because only two Architectonicidae species, *Architectonica perspectiva* (FJ917269) and *Philippia lutea* (AY296843), had COI sequences available in GenBank, we also included four species belonging to two superfamilies assumed closest to Architectonicoidea (Dinapoli and Klussmann-Kolb, 2010): Omalogyroidea (*Omalogyra fusca* - FJ917272, *Omalogyra* sp. - FJ917273) and Valvatoidea (*Valvata piscinalis* - FJ917267, *Cornirostra pellucida* - FJ917282). For the alignment of *16S* rRNA sequences, 50 haplotypes of Architectonicidae sp. (MH557974 to MH558023) were available in addition to one sequence each from *A. perspectiva* (FJ917251), *A. maxima* (KP252986) and *Psilaxis radiatus* (AY081999). Prior to reconstruction of the phylogeny, alignments were trimmed to the shortest region with sequence data for all individuals. Because the available COI sequence for *P. lutea* (623 bp) is from a different section of the gene than *A. perspectiva* (577 bp) and the four other species used to construct the phylogeny, there was only 322 bp of overlap for all sequences. Therefore, *P. lutea* was included in alignments to determine pairwise divergences compared with *A. karsteni*, but it was removed from the phylogenetic tree construction. The resulting alignments were used to construct the respective phylogenetic trees with PhyML 3.0 (Guindon et al., 2010) Geneious plugin, with the following settings: bootstrap replicates = 1000, optimize = Topology/length/rates, Topology search = NNI, nucleotide model substitution = GTR. Significant bootstrap values (≥90) are reported at the nodes.

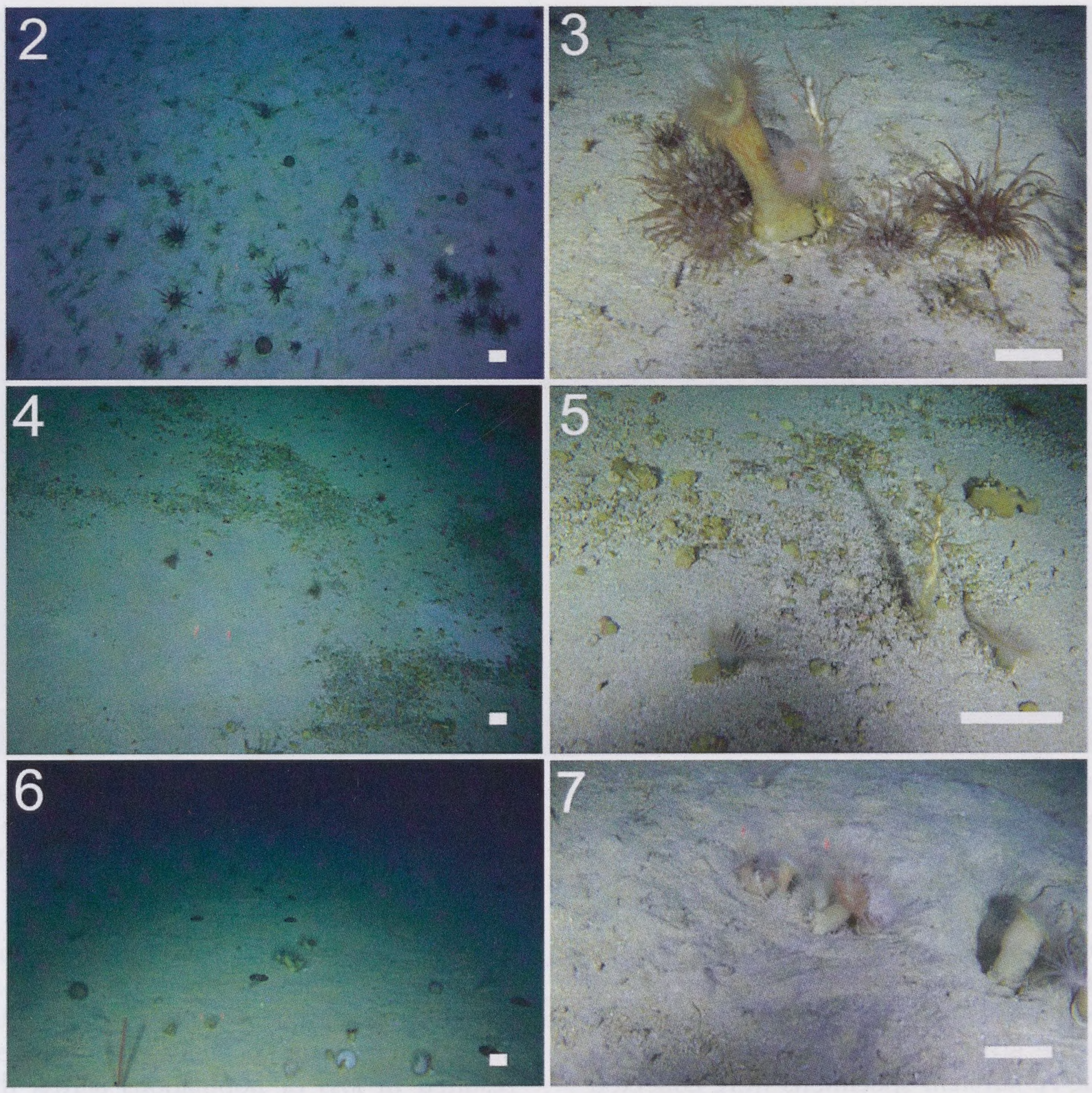
RESULTS

Samples and Habitat Description: A total of seven empty shells and three living *Architectonica karsteni* specimens were collected at four stations within the NDMP (Table 1); however, live specimens were only collected from seamount SF.5 (Figure 1). The smallest shell measured 14.3 mm in diameter and the two largest measured 28.4 mm in diameter. Although 4 to 15 other mollusk species were found at each of the four collection sites, species that co-occurred with *A. karsteni* at three or more sites were *Atrimitra isolata* (Sellanes and Salisbury, 2019) (Mitridae) and *Chryseofusus kazdailisi* (Fraussen and Hadorn, 2000) (Fasciolariidae), which were collected at SF.5, SF.6, and SF.9 seamounts; as well as empty shells of the bivalve *Tucetona kauaia* (Dall, Bartsch, and Rehder, 1938) (Glycymerididae), which were collected at SF.5, SF.9 and S21 seamounts. Of the four seamounts in which *A. karsteni* was collected, three (SF.6, SF.9 and S21) were also studied via ROV surveys. Benthic habitats at SF.6 and SF.9 were dominated by coarse sand and the presence of maërl-rhodoliths, and scattered rocky outcrops were also spotted at these sites (Figures 2–5). The bottom at S21 was dominated by finer sand (Figures 6–7). Wind and current conditions prevented the deployment of the ROV at SF.5, where the live specimens were collected.

Shell Morphology: Shells of live collected specimens have the characteristic yellowish-red marbled color pattern of the species (Figures 8–9) (Bieler, 1993). Subsutural and peripheral ribs are whitish with more or less irregular brown blotches (about 10–16 on 4th whorl of upper peripheral rib, each 1–2 nodules wide); mid ribs are light-brown or bluish-grey, weakly mottled with brown; the basal field is light-brown or bluish-grey with 5–6 dotted or relatively solid spiral lines (marking the reduced spiral ribs of the basal field), wider towards the umbilicus. The umbilical carinae are whitish with a light- to dark-brown marbled pattern. In contrast, most of the empty shells showed a uniform greenish yellow color due to loss of color and different degrees of erosion, although all of them were distinguishable as *A. karsteni* because the distinctive architecture of the shell was sufficiently conserved for species assignment.

Shell morphology of all samples were consistent with the description published by Bieler (1993). Only one specimen had its protoconch measured, because the protoconch of most of the other specimens showed a high degree of erosion, precluding accurate measurements. Even in the photographed specimen, it was difficult to distinguish the boundary between the end of the protoconch, the "stage of arrested growth" and the beginning of the teleoconch (Figure 11). The protoconch of specimen SCBUCN 6928a measures 943 μm (Figure 11) and is whitish to light-brown in color.

COI and 16S rRNA Data: The only sequence data available in GenBank for Architectonicidae species are COI, 16S, and nuclear 18S and 28S rRNA genes for *Architectonica perspectiva*: 16S for *A. maxima*; COI, 28S, and nuclear Histone 3 (H3), U2 and small nuclear RNA for *Philippia lutea*, 16S for *Psilaxis radiatus* and H3 for *Heliacus variegatus*: At the level of uncorrected nucleotide substitutions, *A. karsteni* was 18.6% divergent from both A. *perspectiva* and *P. lutea*. However, the amino acid sequence of *A. karsteni* was identical to that of *A. perspectiva*, whereas these species differed from *P. lutea* by 3 amino acids (2.80%). The percentage divergence based on nucleotide substitutions was 17.63% across the complete 573 bp alignment of *A. perspectiva* and *A. karsteni* and the translated amino acid sequence differed by one (0.52%) change of Val to Ile. Divergence based on nucleotide substitutions was 17.98% across the 623 bp alignment of *P. lutea* and *A. karsteni* and the amino acid sequences differed by six positions (2.90%). The phylogenetic tree reconstructed based on the 580 bp COI alignment shows *A. karsteni* sister to *A. perspectiva* and Architectonicoidea sister to Omalogyroidea, which together are sister to Valvatoidea with a strong (>98) bootstrap support

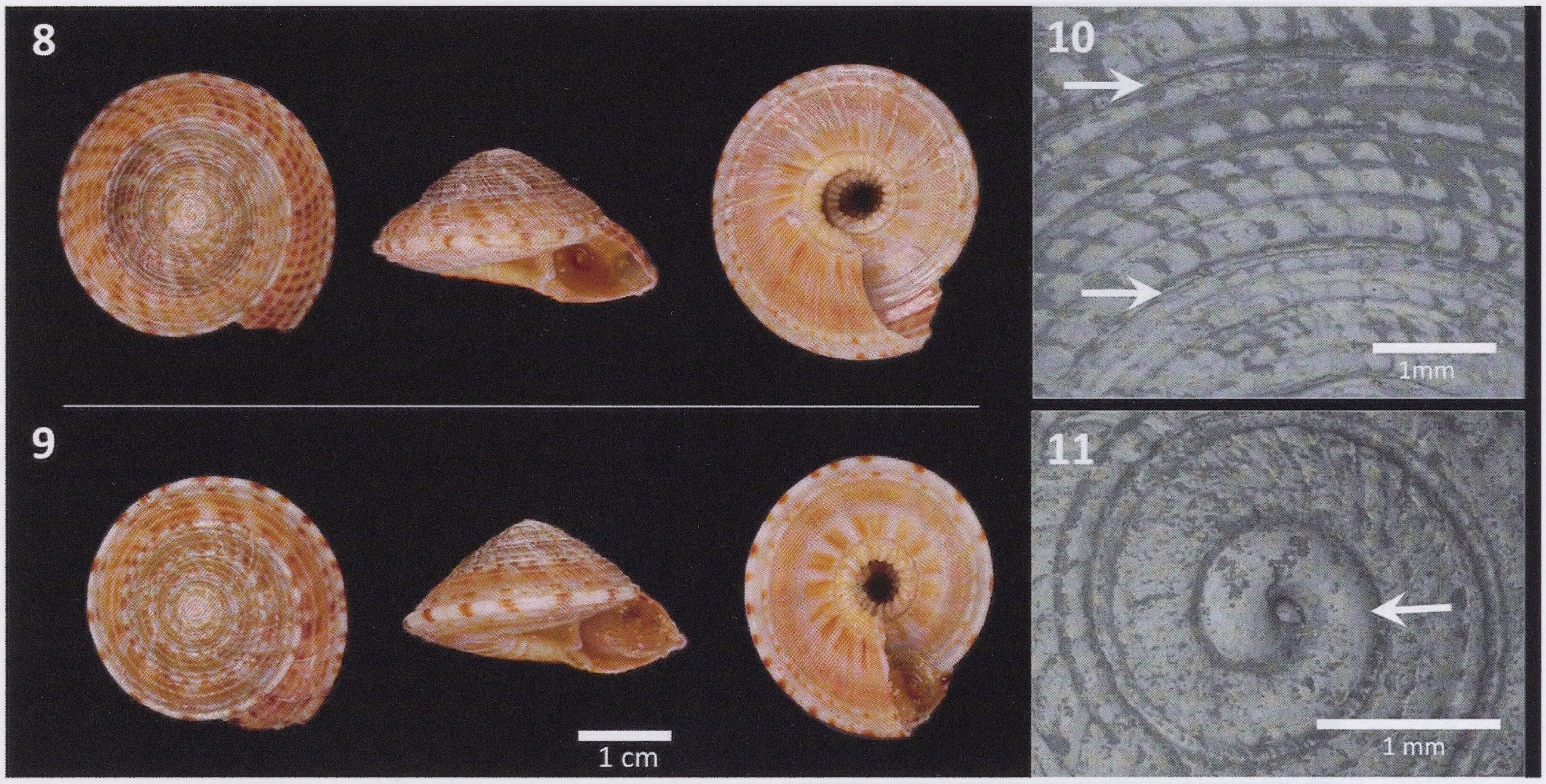


Figures 2–7. Panoramic and zoomed-in images taken with an ROV at the summit seamounts where *Architectonica karsteni* was found. **2.** Panoramic view of SF.6, illustrating the continuous homogeneous bottom of coarse sand with little relief and dominated by sea urchins (*Scrippsechinus fisheri*) and sea anemones (Cerianthidae). **3.** Zoomed in view of sea anemones (*Hormathia* sp. and Cerianthidae) and polychaete tubes (*Eunice* sp.) on SF.6. **4.** Panoramic view of SF.9, illustrating the continuous homogeneous bottom of coarse sand with little relief and clusters of maërl-rhodoliths, dominated by sea anemones (Cerianthidae). **5.** Zoomed-in view of sea anemones (Cerianthidae), polychaete tubes (*Eunice* sp.) and sponges on SF.9. **6**. Panoramic view of S21, illustrating the presence of sea anemones (*Hormathia* sp.), sea pens (*Protoptilum* sp.), sand dollars (*Clypeaster isolatus*) and sea urchins (*Scrippsechinus fisheri*) on the continuous homogenous sandy bottom. **7.** Zoomed-in view of sea anemones (*Hormathia* sp.) on S21. Scale bars = 10 cm. Photo credits: Mathias Gorny /Jan M. Tapia.

(Figure 12). The 415 bp alignment and phylogenetic tree based on 16S rRNA sequence data revealed that none of the other Architectonicidae spp. had the same haplotype as *A. karsteni*, which differed by at least 17.28% from the most similar haplotype (Architectonicidae sp. - MH557975); *A. karsteni* was 19.53% divergent from *Psilaxis radiatus*, the most similar haplotype belonging to a specimen assigned to species level. Because most of the GenBank sequences belong to indeterminate species of Architectonicidae, little additional information can be extracted from the resulting phylogenetic tree (Figure 13).

DISCUSSION

Information about the invertebrate benthic fauna inhabiting seamounts of the Salas y Gómez and Nazca Ridges is scarce, and most of it is associated with studies carried out between 1973 and 1987 (Mironov and Detinova, 1990; Parin et al., 1997). Parin et al. (1997) reviewed the fauna for 22 seamounts, from which they reported one species of Polyplacophora, 27 species of Gastropoda (most of them of the superfamily Conoidea), seven species of Bivalvia, and seven species of Cephalopoda, the latter probably pelagic. No representatives of the family Architectonicidae were reported for the area in the studies reviewed by these authors or any other study until now. The new records of *A. karsteni* in the NDMP provided as part of this study not only adds a species to the malacological fauna reported for the Salas y Gómez and Nazca Ridges, but also restores a species to Chile that has been believed extinct from its jurisdictional waters since 23–17 Ma (Nielsen and Frassinetti, 2007; Nielsen and Glodny, 2009; Finger et al., 2013).



Figures 8–11. *Architectonica karsteni* sampled in Desventuradas islands (–25.4272°,–81.8806°). **8–9.** Dorsal, lateral and ventral views of specimens 6928a and 6928b (scale bar = 1 cm). **10.** Teleochonch and **11**. protoconch (scale bar = 1mm, SEM) sculpture of SCBUCN 6928a. Arrows in **10** indicate whorl border sutures and the arrow in **11** shows the border of the protoconch.

The presence of *A. karsteni* in the seamounts of NDMP may result from two alternative scenarios. The first is that the sampled individuals could belong to relict populations that persisted in the area since the Miocene. The seamounts where the *A. karsteni* specimens were collected have ages between 32 and 34 Ma (EarthRef.org, 2019) and likely hosted active reefs on their summits during the Miocene, as indicated by fossil corals and gastropods found on nearby seamounts (Menard, 1964; Gevorkyan et al., 1987). After oceanographic changes occurred that resulted in loss of populations across their geographic range, the relict populations of *A. karsteni* could have remained isolated without undergoing changes in shell morphology, which would be consistent with evidence suggesting that Architectonicidae of the Indo-Pacific is a slowly evolving group (Bieler, 1993). Bieler based this conclusion in part on the lack of morphological differences found between Indo-Pacific and Atlantic species despite the genetic flow between them ceasing with the closure of the Isthmus of Panamá in the Pliocene (~3.5 Ma). It has been suggested that the Humboldt Current System, with its characteristic cold and nutrient-rich waters, could act as a barrier, at least, separating the biota of this area from the South American coast (Friedlander et al., 2016). Moreover, seamounts are also known to generate particular circulation patterns (e.g., Taylor column, Taylor cone) that could contribute to the retention of locally generated larvae (Rogers, 1994; 2018). All these physical factors could contribute to the isolation of the local fauna, resulting in the populations of seamounts being repopulated with larvae generated within the nearby seamount populations and under complete isolation since 23 mya (Nielsen and Frassinetti, 2007). The oceanographic changes registered since the Miocene along the coast may not have affected the seamounts but may have isolated the seamount populations of *A. karsteni* from the continental coasts. If these isolated populations

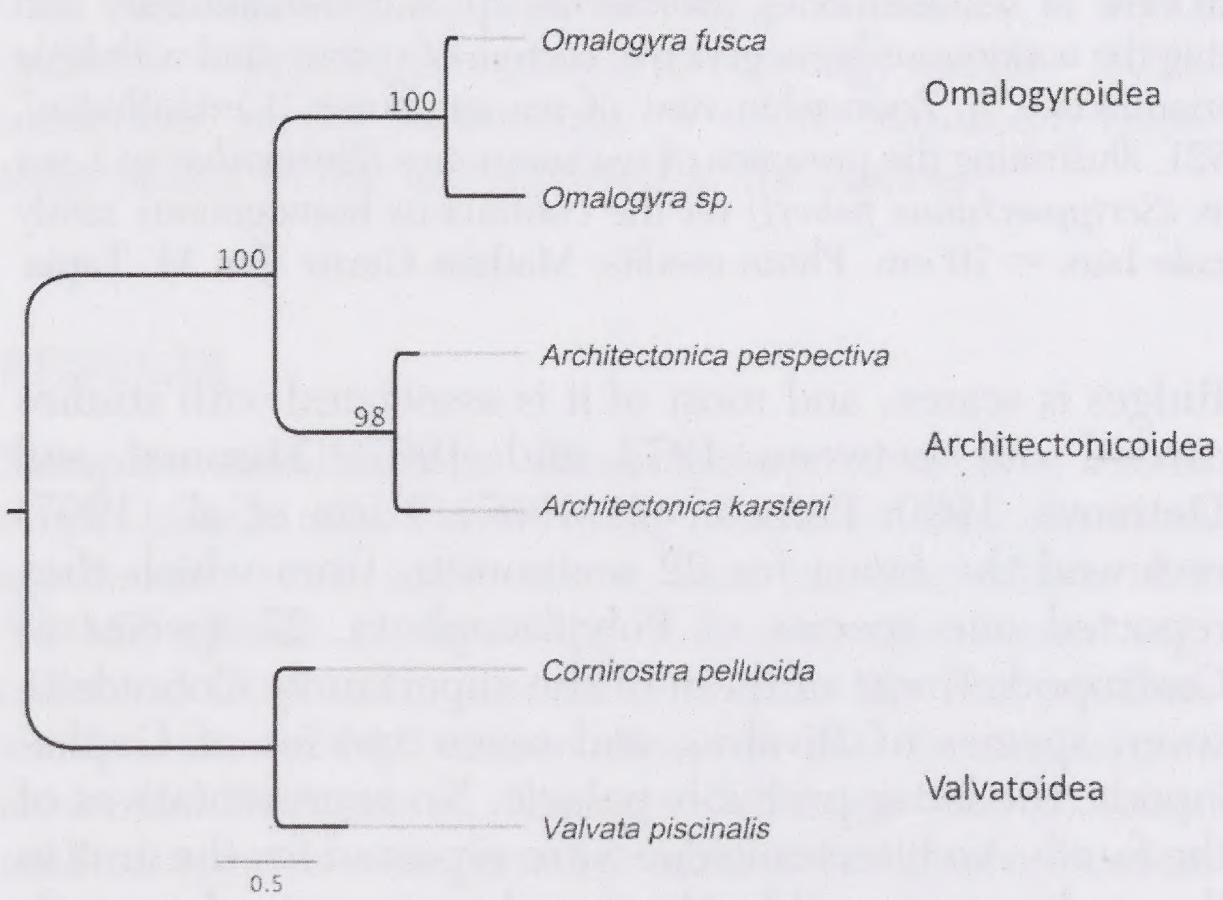


Figure 12. Maximum-likelihood inferred phylogenetic reconstruction based on 580-bp alignment of partial *COI* sequences of species of Architectonicoidea (*A. karsteni* SCBUCN 7066, Accession GenBank # MN270389 and *A. perspectiva* - FJ917269), Omalogyroidea (*Omalogyra fusca* - FJ917272, *Omalogyra* sp. - FJ917273) and Valvatoidea (*Valvata piscinalis* - FJ917267, *Cornirostra pellucida* - FJ917282). Only bootstrap values above 90 are shown.

Figure 13. Maximum-likelihood inferred phylogenetic reconstruction based on 415 -bp alignment of partial 16S rRNA 16S sequences of 50 haplotypes of Architectonicidae sp (MH557974 to MH558022), *Architectonica maxima* (KP252986), *A. perspectiva* (FJ9117251), *A. karsteni* (MH270388) and *Psilaxis radiatus* (AY081999).

continued to evolve genetically with little changes in shell morphology, *A. karsteni* found on the NDMP seamounts could be a cryptic (and possibly sibling) species of populations currently living on the continental shelf of the Pacific coast of Central America, as documented for several sacoglossan sea slugs species (Carmona et al., 2011). The fact that *A. karsteni* has not been sampled from other seamounts is not attributed to the mesh size used in other studies, since gastropod species with similar or even smaller sizes have been reported (e.g., *Ptychosyrinx naskensis* Sysoev and Ivanov, 1985). Neither is it likely related to differences in depth of the seamounts sampled, because the depths of the seamounts sampled during the CIMAR 22 (Table 1) were in the same range as previous expeditions (Parin et al., 1997). Although we still cannot rule out insufficient sampling effort, the absence of *A. karsteni* in other seamounts may have been due to the existence of differences in the oceanographic conditions and subsurface marine currents that may affect the subsistence of this species directly, or indirectly through effects on their cnidarian preys.

In the second scenario, the sampled population could be the result of colonization of these seamounts after the extinction of *A. karsteni* from this latitude during the Miocene. Architectonicids are known to produce teleplanic larvae able to drift in near-surface currents (Scheltema, 1968, 1971; Scheltema and Williams, 1983) and to delay metamorphosis; thus, their larvae can disperse over great distances (1,000's of km under suitable current conditions). In this scenario, the sampled populations would represent recolonization events of this region. Gene flow could exist between populations of the NDMP seamounts and the continental shelf of the Pacific coast of Central America and both populations would be thus be part of a metapopulation.

Addressing the history of these populations would thus require appropriate sampling methods to clarify the entire range of the species and to obtain samples suitable for detailed morphological and genetic analysis. Although the systematic position, based on anatomical characters, and complicated taxonomic history of Architectonicidae have been discussed by Haszprunar (1985, 1988) and Bieler

(1988, 1992), little attention has been paid to the genetic differences that may exist within and among Architectonicidae species. Therefore, few sequences are available for addressing phylogenetic relationships within Architectonicidae and to assess issues of historic and modern population connectivity. Not enough genetic data are available, and we did not have access to sufficient samples of *A. karsteni* to determine whether the sampled populations represent relict populations, cryptic species, or a recent colonization of modern *A. karsteni.* Accordingly, we provide barcode data from one of our specimens as an aid to future studies. To date, only 63 partial gene sequences are available in GenBank, 50 of which are larval 16S rDNA sequences from specimens that were not identified below family. Interestingly, only two COI sequences are available for Architectonicidae, despite this genetic marker being widely used for DNA barcoding of marine species (Bowen et al., 2014). The barcode data provided here were used to confirm the phylogenetic placement of this species relative to Architectonicidae with published 16S and COI sequences. Although the COI sequences *A. perspectiva* (FJ917269) and *P. lutea* (AY296843) had a similar percentage of mutations compared with *A. karsteni*, *P. lutea* presents a higher number (6) of non-synonymous mutations across a similar number of base pairs (623 vs 573 bp). In addition, the 16S rRNA data supports the position of our *A. karsteni* specimen as belonging to the genus *Architectonica*, of which *A. perspectiva* is the type species. Thus, this genetic data from the NDMP specimens are consistent with our classification of *A. karsteni* based on the morphological characteristics. To further elucidate the taxonomic status of this NDMP population and to assess potential crypsis, future studies should address a more representative genetic sampling of the group, including specimens throughout the entire distributional area.

CONCLUSIONS

Until now, the malacofauna of Salas y Gómez and Nazca Ridge seamounts is considered to have a high affinity with the Indo-Pacific fauna (Parin et al., 1997). However, a recently described species, *Atrimitra isolata,* seems to have morphologic affinities with species from the southeast Pacific coast (Sellanes et al., 2019). Similarly, we report a species that was present at the coasts of continental Chile during the Miocene. This new record of *A. karsteni*, together with the discovery of the above mentioned new species in NDMP, highlights the need for more studies of the region, which not only focus on biodiversity but also on the phylogenetic relationships between them and the fauna of surrounding areas.

ACKNOWLEDGMENTS

For their assistance at sea, we would like to thank the Captain and crew of R/V CABO DE HORNOS of the Chilean Navy, and the scientific personnel participating in the CIMAR 22 cruise. Special thanks to Jan M. Tapia and Jorge Avilés for their help during collection, handling and curation of the specimens. We are grateful to Dr. Matthias Gorny from OCEANA who piloted the ROV that obtained the images of the habitat at the seamounts surveyed in this study. Thanks to Jan M. Tapia for the ROV photos. CMA thanks Dr. Rubén J. Díaz for his unconditional support and for encouraging her to continue in the race. Suggestions by Sven Nielsen and an anonymous reviewer greatly helped to improve the manuscript. Funding was provided by grants CONA C22 16-09, FONDECYT 1181153 and FONDEQUIP EQM150109 to JS; New Hampshire-INBRE through an Institutional Development Award (IDeA), P20GM103506, from the National Institute of General Medical Sciences of the National Institutes of Health; and Millennium Nucleus ESMOI (NC120030).

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A history of eastern Pacific marine heterobranch research

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I.

"The Mollusks whose history and characters it is the purpose of the present work to illustrate, form an attractive group of the class Gasteropoda, until lately little noticed, and supposed to be of small extent, but which modern researches have brought into more prominent importance."
Joshua Alder and Albany Hancock, 1845,
A Monograph of the British Nudibranchiate Molluscs

We live in one interconnected global ecosystem, from the butterflies in the Amazon to the polar bears on the Arctic ice. Because of radical anthropogenic activities, today the tropical rain forest is burning and the polar ice caps are melting. Earth's health is in danger. The need for good science, historical perspectives, and rational decisions is even more imperative. Contributions to our knowledge of the biodiversity in any faunal region form a significantly useful aspect in our search for understanding and preserving life on earth.

By its nature, in terms of both the observers and the observed, science is an international endeavor (Gosliner, Cervera, and Ghiselin, 2008). Science is without borders. This is especially demonstrated by the history of "opisthobranch" research in the northeastern Pacific. From Alaska to Peru, these animals have distributions spanning multiple countries on both sides of the northern Pacific. The investigators have been equally international. For instance, Johann Eschscholtz, from modern Estonia, participated in two circumglobal expeditions in the early 19th century, collecting in regions that flew different flags than today. From Spanish and Mexican California, and Russian Alaska, he named a nightshade plant, a tenebrionid beetle, two salamanders, a sand dollar, and two nudibranchs. The Danish physician Ludwig Sophus Rudolph Bergh named a number of Alaskan nudibranch species based on specimens collected by the U.S. malacologists William Healey Dall and William Simpson, including the still-valid species of nudibranch *Dendronotus dalli*, which he described only from the pharyngeal bulb of an unknown animal! Today such international cooperation includes co-authorships, sharing of specimens, and joint research expeditions. Especially notable are DNA phylogenies involving collaborating researchers from multiple countries.

Science builds on both the research results and the investigative methods and publication styles of previous generations. Compendia and monographs of taxonomic or geographic groups were, and are, the technical equivalents of the modern field guides. During the 18th and 19th centuries, numerous volumes on conchology were published, highlighted by the works of British natural history aficionados such as the Rev. John Lightfoot (1786), the Sowerbys I and II (1821–1875), Lovell Augustus Reeve (1841–1860), and the Rev. Philip Pearsall Carpenter (1855–1872). These guides to almost-random geographic areas, were based on shell collections in British cabinets. Apparently dead sea shells are more attractive and collectible than pickled sea slugs!

The first comprehensive monographs on the nudibranch fauna of a region (with color illustrations of living animals) was Joshua Alder's and Albany Hancock's magnificent series on their own British Isles (1845–1855). Later compendia were a mix of national and international efforts. Especially significant (among many others) were Bergh's (1905) German report on the SIBOGA Expedition to the Netherlands' East Indies Colonies, Charles Norton Edgecombe Eliot's studies in the Indian Ocean (1902–1916), Kikutaro Baba's (1949, 1955) descriptions of specimens collected by His Majesty the Emperor of Japan, in Sagami Bay, and Jean Risbec's (1956) work on Vietnamese species.

Myra Keen's second edition of *Sea Shells of Tropical West America* (1971) included a section on nudibranchs. Based on a species list compiled by H. Bertsch, Wesley Farmer and Steven Long, the overview was written by James Lance. It included brief descriptions of 72 species, notes on their habitats and distribution, and four plates of color photographs of the living animals.

In 1976, Thomas E. Thompson and Gregory H. Brown published a guide to the British opisthobranch mollusks, with detailed drawings and anatomical and life history information. Schmekel and Portmann's (1982) guide to Mediterranean opisthobranchs included magnificent full-

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color paintings of each species by Ilona Richter, along with comprehensive anatomical, biogeographic, and taxonomic data.

Field guides have played an important role in expanding access to natural history information and identification of species' groups for "citizen scientists." First popularized for birds (e.g., Peterson's Identification System of the 1930s), these works now cover almost all aspects of the biological, geological, and chemical realms on and off the planet. Of course, the nudibranchs began to receive attention.

The first field guides, illustrated completely by full-color photographs of living opisthobranchs, appeared within about a year's span. McDonald and Nybakken (1980) published a guide to the nudibranchs of California (with 112 species). They included a dichotomous key for identification of the organisms and an aquarium photo of each species. Dave Behrens (1980), in the first edition of his book, published a comprehensive guide to all the known 162 heterobranch species in the northeast Pacific, from Alaska to the southern tip of the Baja California peninsula. Bertsch and Johnson (1981) documented 78 species from the Hawaiian Islands, often presenting multiple in situ photographs of an animal's life history. For the far western Pacific, John Orr (1981) published aquarium photos of some 50 species of Hong Kong nudibranchs, representing just a part of what he considered to be a far more extensive fauna.

Since these works, and with the increased popularity of underwater macro-photography by scuba divers, numerous nudibranch field guides have appeared in multiple languages for various parts of the world's oceans: e.g., Arctic and Russian Seas (Martynov and Korshunova, 2011), the Indo-Pacific (Gosliner, Behrens, and Valdés, 2018), Korea (Koh, 2006), Bali and Indonesia (Tonozuka, 2003), Australia's Great Barrier Reef (Marshall and Willan, 1999), Mexico (Hermosillo et al., 2006), the Caribbean (Valdés et al., 2006), and Brazil (García-García et al., 2008). The list keeps expanding!

Multiple conferences dedicated to sea slugs have been organized (Malaquias et al., 2011). Since its inception, the Western Society of Malacologists has presented near-annual opisthobranch symposia at its meetings, with international participation (Figures 1–3). The International Workshop of Opisthobranchs, now the International Heterobranch Workshop (!) has been held at various sites around the world (Figure 4).

With the wealth of information accessible electronically, there are highly useful web sites dedicated to sea slugs, most notably Bill Rudman's Sea Slug Forum (not currently active, but archives are available at www.seaslugforum.net), and Mike Miller's Slug Site (now in its 21st year, found at www.slugsite.tierranet.com), with ongoing weekly postings. Especially significant is Gary McDonald's comprehensive *Bibliographia Nudibranchia.* Numerous underwater photographers post brilliant photographs on their personal sites. The change from film to digital cameras has helped greatly to revolutionize nudibranch imaging.

Indeed, today—as Alder and Hancock observed nearly two centuries ago—sea slugs have been "brought into more prominent importance" than even those prescient investigators would have imagined.

This work is a human and scientific history, describing the individual scientists and the development of our

Figure 1. Eveline Marcus (left) and Louise Schmekel, Monterey, California, July 1986. (Photo by Hans Bertsch)

Figure 2. Slug Talk. Presentation announcement of opisthobranch workshop, Seattle, Washington, August 2006. (Drawing by Jan Kocian)

Figure 3. Opisthobranch workshop participants, joint meeting of the Western Society of Malacologists (WSM) and the American Malacological Society (AMS), Seattle, August 2006.

understanding of the marine heterobranch fauna from the eastern Pacific, from the southern limit of the Panamic Province to the Arctic shores of Alaska. Although numerous, the terrestrial species of Heterobranchia (land snails and slugs) are not included. To establish a wider context, various early voyages of exploration along the Pacific coast are mentioned, along with broader, non-heterobranch molluscan studies. It should be noted that many of the pioneering naturalists published on a wide variety of disparate taxa across phyla and even kingdoms (Bertsch, 2015). Recent taxonomic changes based on DNA analyses have completely revised the traditional classification schemes. Although Opisthobranchia is no longer considered a valid monophyletic taxon, the term is used throughout this work in its historical sense.

II.

"A number of times we were asked, Why do you do this thing, this picking up and pickling of little animals? Finally we learned to know why we did these things. The animals were very beautiful. Here was life from which we borrowed life and excitement. In other words, we did these things because it was pleasant to do them.... Here was no service to society, no naming of unknown animals, but rather–we simply liked it."
John Steinbeck and E. F. Ricketts, 1941,
Sea of Cortez

Based on the publications of investigators, one can identify seven historical periods of "opisthobranch" research in the eastern Pacific. Until the recent explosion of nudibranch interest and researchers, most of the periods are represented each by only a handful of persons. Authorship of a publication is only the beginning of a historical perspective. Who were these researchers? What were their meanderings? What was the scope of their scientific investigations? The international character of the research has been a constant. Today's known eastern Pacific fauna consists of species with type localities around the world, described by researchers of multiple nationalities. This chapter describes key aspects of these investigators' lives and their endeavors.

During these periods there have been significant developments in our knowledge of the taxonomy and natural history of the marine heterobranchs from this region. There has been a dramatic change in the numbers of species named (and the taxa to which they belong) before and after 1961. Before 1961, the Nudipleura constituted only 60% of the known heterobranch species. Today, however, they make up 75% of all known marine heterobranch species in the eastern Pacific. In the 200 years from 1761–1960, 104 species of Nudipleura were named, contrasting with 72 species of the non-nudipleuran heterobranchs. During the 60-year span from 1961–2019 the numbers of nudipleura species named increased to 168 but decreased to 25 for the non-nudipleuran heterobranchs (Figure 5). The total numbers of species and the average numbers named per year showed several patterns. The average number of nudibranchs named increased dramatically from 0.5 to over 2.5 species named per year before and after 1961. However, the yearly average of species named among all the other Heterobranchia (many of these are the shelled forms) remained the same, less than 0.5 (Figure 6). Looking at each of the seven periods described below, one sees a near-constant annual number of non-nudipleuran heterobranchs named, but a significant increase in the numbers of species of

Figure 4. Participants at the 4th International Workshop on Opisthobranchs, meeting of the WSM, University of California, Santa Cruz, June 2012.

Nudipleura named per year (Figure 7). Because the periods vary in length, the average named per year is more indicative of the research efforts and shows more precisely the pronounced increase in the naming of Nudipleura species (Figure 8).

1. Pre-1860: From Indigenous Peoples and Foreign Visitors to Gould

Indigenous people living on the Pacific coast of North America have been well acquainted with mollusks, including Heterobranchia, for centuries. Mollusks are dominant components of midden mounds along the shores of the eastern Pacific (e.g., Tellez-Duarte et al., 2001, upper Gulf of California; Glassow, 2010, Channel Islands; and Trant et al., 2016, British Columbia). Numerous shelled gastropods have been found in these "trash dumps," but no marine heterobranchs have been recorded from any of them. Therefore, any knowledge, use, or consumption of sea slugs by indigenous nations can only be documented by ethnohistorical methods. The oft-cited report of *Tritonia tetraquetra* being named *"Tochui,"* and consumed by the Kuril Island people (Pallas, 1788) is probably based on a linguistic misinterpretation. The similar-sounding Ainu word *togoi*, means invertebrates or mollusks in general, which were consumed, but not specifically the

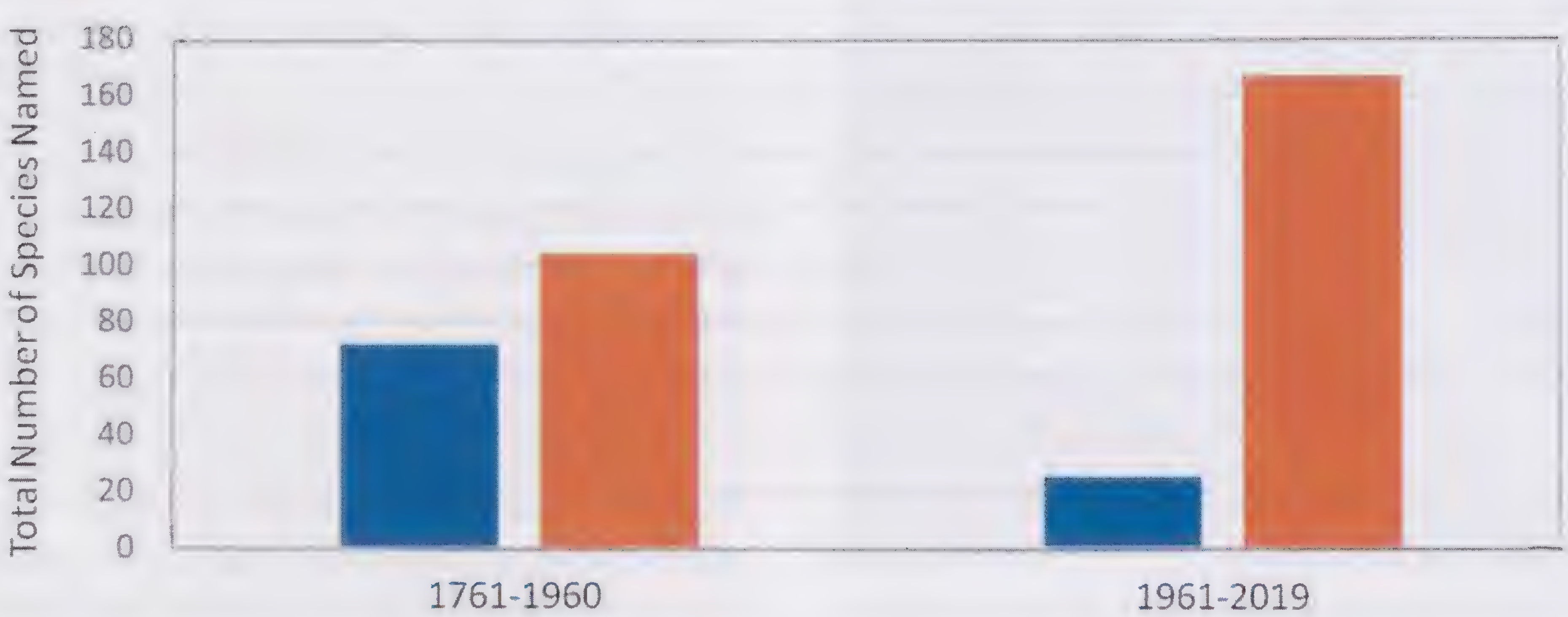

Figure 5. Numbers of pre- and post-1961 descriptions of eastern Pacific Heterobranchia species. Orange: Nudipleura. Blue: Non-Nudipleura Heterobranchia.

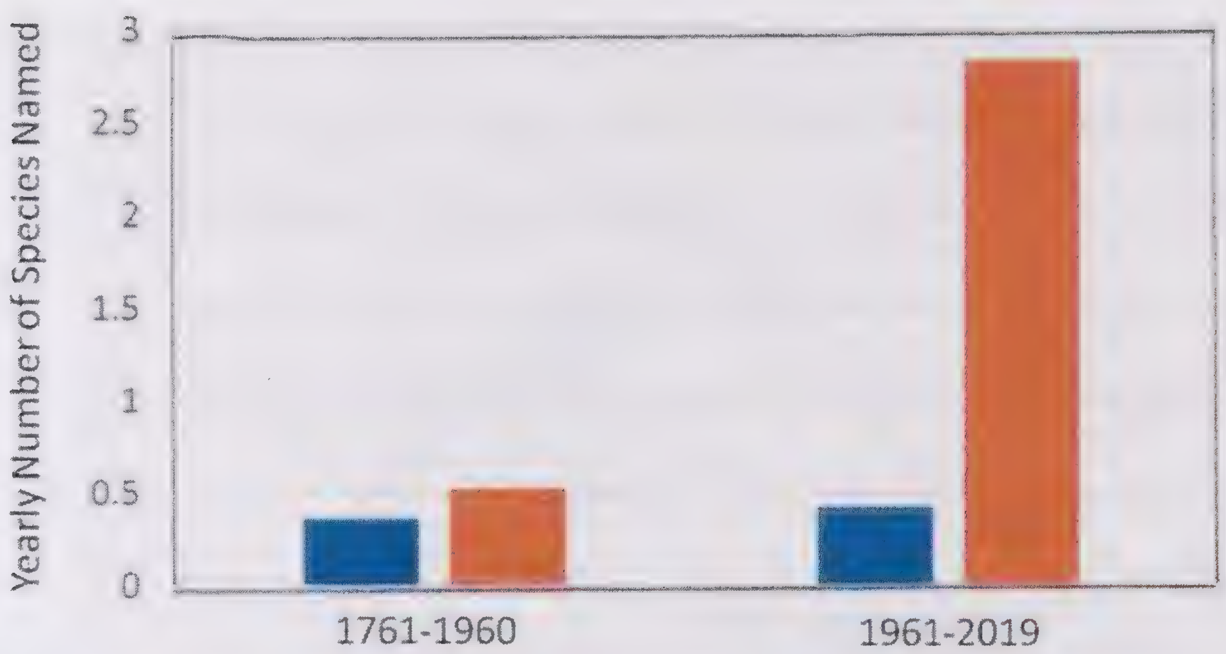

Figure 6. Annual number of eastern Pacific Heterobranchia species named pre- and post-1961. Colors as in Figure 5.

deep-water tritoniid nudibranch (Martynov, pers. comm., 11 September 2011).

For centuries, the Comcaac (Seri) of Sonora have been gathering mollusks for food, games, and decorative and medicinal uses along the eastern shores of the Gulf of California. They knew about and used sea slugs, even giving common names to four species. This rich ethnohistorical cultural knowledge has been passed on to the present generation (Marlett, 2014: 168–171). *Bulla gouldiana* is called *cacaapxon*, "what fattens something." Living animals of *B. gouldiana* were played with by children who shot them like marbles, and the sticky animal would sometimes adhere to the target. Empty shells were strung in necklaces or used as dangling ear ornaments (Bertsch and Marlett, 2011). Found on shallow sand flats, *Navanax inermis* was aptly named *hant iti queemij*, "what moves slowly on land." *Aplysia californica*, occasionally used as bait, was given the name *hatx cöcazoj*. The etymology is unclear, but *hatx* refers to rump or buttocks. The color purple was even named "the ink of *hatx cöcazoj*." The blood-colored ink of *Aplysia* caused precautionary warnings that pregnant or menstruating women not touch it. Finally, *Berthellina ilisima* is named *xepenozaah*, "sun in the sea." Imagine rolling rocks in the intertidal zone under the glaringly hot Sonoran desert sun and finding this orange blob!

With the exception of Eschscholtz, prior to 1860, marine heterobranch species now known to occur in the eastern Pacific were named scientifically from specimens collected from other regions, or by investigators who were not working in this region. For instance, *Aeolidia papillosa* (Linnaeus, 1761) was first reported from the northeastern Atlantic, *Dolabella auricularia* (Lightfoot, 1786) was based on an internal shell collected in the Indo-Pacific, and the type material of the previously mentioned *Tritonia tetraquetra* (Pallas, 1788) was collected from the Kuril Islands, in the northwest Pacific.

The publication of *Eolidia* (now *Fiona*) *pinnata* and *Cavolina* (now *Hermissenda*) *crassicornis* by Eschscholtz in 1831 first named a nudibranch species from a type locality (Sitka, Alaska) in the eastern Pacific (Figure 9). Johann Friedrich von Eschscholtz (1793–1831) served as surgeon and naturalist on two circumglobal Russian expeditions commanded by Otto von Kutzebue, on board the RURIK (1815–1818) and the PREDPRIAETIE (1823–1826). His zoological results of these journeys described species across a wide assortment of taxa. In addition to the previously mentioned species, he named the marine turtle *Lepidochelys olivacea* (Eschscholtz, 1829) based on specimens from Manila and Sumatra, and King Kamehameha's butterfly *Vanessa tameamea* (Eschscholtz, 1821) from Hawaii. After his voyages, Eschscholtz returned to teaching full-time as Professor of Anatomy at the University of Dupat.

During the first half of the nineteenth century, other sailing expeditions brought additional specimens from around the world back to Europe to be named. For example, the French voyage of the ASTROLABE (1826–1829), commanded by M. J. Durant d'Urville, brought back shells of tropical aplysiids that were named by Quoy and Gaimard (1832). These military and scientific expeditions spurred the United States to finally launch its own national exploring survey. Although the planning stages were plagued by personal jealousies, and political and financial obstacles, a flotilla of six vessels finally departed Norfolk, Virginia, on 18 August 1838, under the command of Charles Wilkes. Over the span of four years and 87,780 miles, this United States Exploring Expedition (U.S.E.E.) crisscrossed, surveyed and dredged the oceans, and even

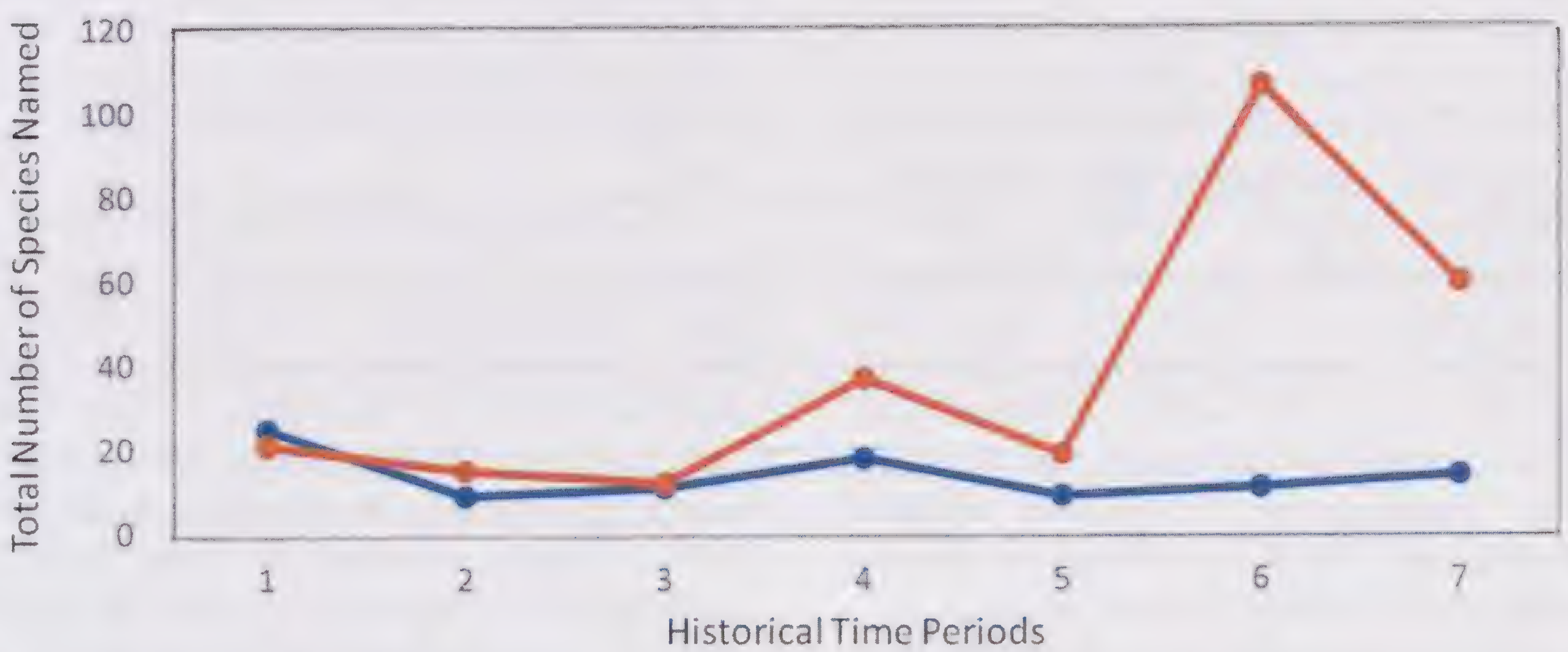

Figure 7. Numbers of eastern Pacific Heterobranchia species named by historical period. Colors as in Figure 5.

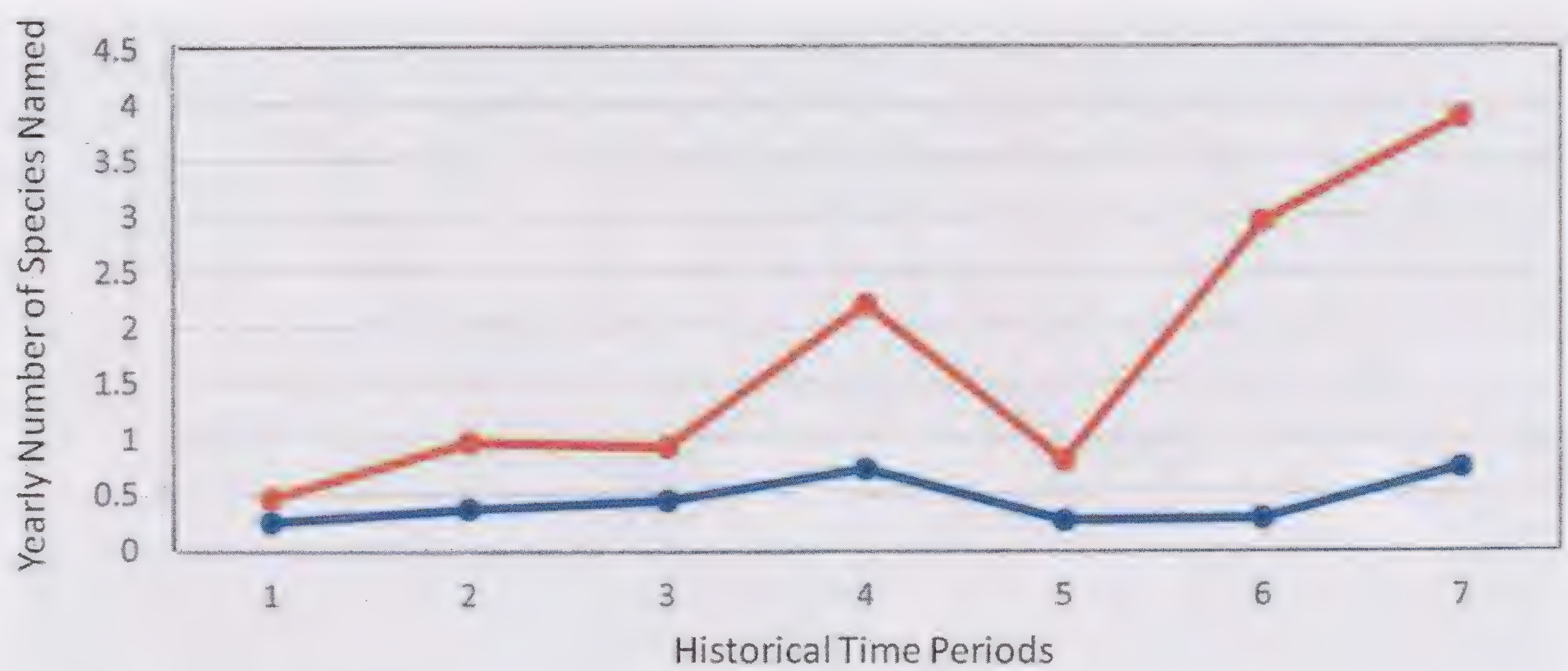

Figure 8. Annual numbers of eastern Pacific Heterobranchia species named per historical period. Colors as in Figure 5.

collected on land and in rivers. Two vessels were lost at sea, and two were deemed unseaworthy. Only the VINCENNES and the PORPOISE sailed the entire voyage. The description of the marine mollusks collected during this expedition was entrusted to Augustus Addison Gould.

Dr. Gould (1805–1866) was a Boston physician, instrumental in the founding and success of the Boston Society of Natural History. As a young college student, he began a lifelong interest in natural history. While practicing as a physician, he spent most of his spare time studying and describing mollusk species. Because of his publications (see Johnson, 1964), especially *Report on the Invertebrates of Massachusetts* (1841), he was chosen to write the mollusk report of Wilkes' expedition. His description of the nudibranch *Melibe leonina* (Gould, 1852) was the first description by an American of a marine heterobranch collected in the eastern Pacific. The type had been dredged at Port Discovery, Puget Sound, by Mr. Dyer of the U.S.E.E. Subsequently Gould (1853, 1855) named four cephalaspideans, two collected by Colonel Ezekiel Jewett at Santa Barbara (*Acteocina cerealis* and *A. culcitella*), and two from San Diego (*A. inculta* and *Haminoea vesicula*) collected by William Phipps Blake. Jewett was an accomplished private collector; he had spent ten weeks in Panama, then journeyed to California, collecting along the coastline from San Francisco to Ventura. Blake had arrived in California finishing a reconnaissance survey for a railroad route across the southwestern United States.

A few years later, Gould's correspondent and colleague, the English-Canadian Presbyterian minister Philip Pearsall Carpenter (1819–1877) named five species of eastern Pacific shelled slugs from Mazatlán, southern California, and Puget Sound (Palmer, 1958). He studied additional material from the collections by Jewett and others that Gould had used. Carpenter's original descriptions of some species were rather terse, e.g., *Volvulella cylindrica* was "like a grain of rice, pointed at one end," and *Rictaxis punctocaelatus* was "Small: grooved with rows of dots: pillar twisted as in *Bullina*." In later publications, he did present more expansive and detailed descriptions.

Prior to 1860 with the exception of Eschscholtz, heterobranchs now known to occur in the eastern Pacific were described from specimens collected in other marine provinces, or by investigators who were not working in this region. This was about to change.

2. 1860–1875: Pioneering Intertidal Naturalists

The second period of eastern Pacific opisthobranch research encompasses the work of three men who were the first scientists actually to live in this region and to name heterobranch species which they had collected.

At the beginning of the century, Eschscholtz had arrived at the shores of western North America by sailing vessel and then returned to his native Estonia to eventually publish his findings in the German narratives of the Kotzebue expeditions. In contrast, James Graham Cooper (1830–1902) traveled by boat and horseback to the U.S. Pacific coast and then stayed there, publishing his observations in English in the *Proceedings of the California Academy of Sciences* (of which he was an early member), and other U.S. journals and scientific government reports. Both men were accomplished field biologists, by land and sea, and skilled writers, describing a diversity of marine invertebrates, terrestrial fauna, and flora. Before settling in central California to earn a living in medicine, Cooper served as a contract surgeon and naturalist on railroad surveys and military expeditions, traversing the continent, exploring both the Atlantic and Pacific coasts and the western deserts and mountains. He was the pioneer of California nudibranch research, being the first to collect and describe species from this coastline (Figure 10; Coan, 1982).

Upon graduation from the College of Physicians and Surgeons in 1851, the young Cooper spent two years practicing medicine at New York City Hospital. He was then appointed physician and naturalist for the 1853–1855 Railroad Survey Expedition (under the command of Captain George B. McClellan, who in 1862 led the Union troops at Antietam) in Washington Territory. When finished, Cooper returned to New York, again by boat, crossing the Isthmus of Panama. He spent the next five years in New York and Washington, D.C., traveling the east coast between New England and Florida, traversing

Figure 9. Original illustrations of the first two nudibranch species named from the eastern Pacific, *Eolidia* (now *Fiona*) *pinnata* and *Cavolina* (now *Hermissenda*) *crassicornis*. (Eschscholtz, 1831: Plate XIX, figs. 1 and 2)

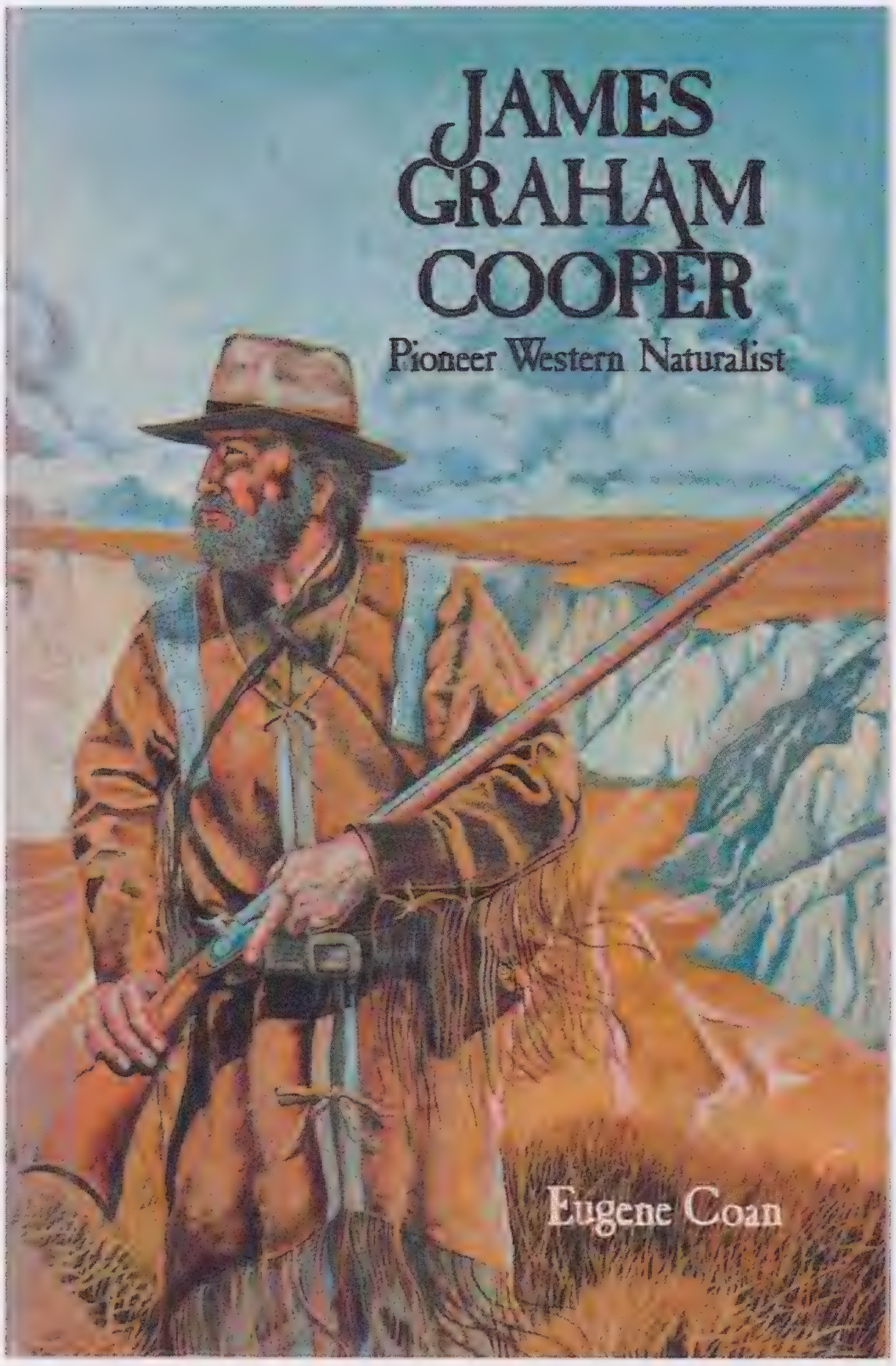

Figure 10. Book cover of James Graham Cooper biography. (Coan, 1982)

the U.S. to Wyoming Territory on the ill-fated 1857 Wagon Road Expedition, and doing research in the major east coast museums. He renewed his family's earlier acquaintance with Spencer Fullerton Baird, the first Curator of the United States National Museum (USNM). It was Baird who recommended him to participate in the 1860 Military Expedition to the West as surgeon and naturalist. This expedition crossed the Rockies through Montana, ending at Ft. Vancouver. From there he went by steamer to San Francisco and then was posted for five months at Fort Mojave, Arizona, on the east bank of the Colorado River. Later he named a tortoise and bird he had collected in the high desert region. When he returned to San Francisco via San Diego, he collected along the coast and the offshore islands. Now firmly ensconced in California, he was hired by Josiah Dwight Whitney as Zoologist of the California Geological Survey, collecting birds, mammals, fossils, and marine organisms throughout the state. The last 40+ years of his life he devoted to medicine, his family, field work, writing and publishing, and serving the nascent California of Sciences in a variety of curatorial and directorial roles.

Dr. Cooper had wide-ranging interests, from geology and paleontology, to phytogeography and ethnology. Although the majority of his publications were on conchology, he also published papers on mammals (see Taylor, 1919) and birds (see Emerson, 1899). His contributions to ornithology were so impressive that when a group of bird enthusiasts organized a study group in 1893, they named it the Cooper Ornithological Club in his honor. In addition to 10 species of bivalves and 89 species of gastropods (E. Coan, pers. comm., December 2019), Cooper named two species of brachiopods, one insect, nine fish, two reptiles, four birds, and a subspecies of mammal. His etymological explanations are sometimes whimsical. He named Lucy's Warbler *Vermivora luciae* in honor of S. F. Baird's daughter, whose "presence, like the bird's, would go far to make a garden of the desert." The desert tortoise *Gopherus agassizii*, which he considered "the emblem of slowness," he named in honor of Harvard's anti-Darwinian professor Louis Agassiz! In naming nudibranchs, he used no patronyms, but dedicated species to a collecting location or a distinctive morphological feature (e.g., *sandiegensis* and *iodinea*). Cooper's 1863 paper described 14 species of nudibranchs (12 are still recognized as valid today) which he had collected from the coast and islands of southern and central California.

Robert E.C. Stearns (1827–1909) went back and forth across the U.S. during his career. He worked as a paymaster for copper mines in Michigan, then moved to California in 1858 as editor of the weekly *Pacific Methodist*. Along with the Unitarian minister Thomas Starr King, his efforts significantly influenced the voters to approve California's statehood entry as a Union, non-slave state. He was a Deputy Clerk of the California Supreme Court, searched for mollusks in southwest Florida with William Stimpson and Ezekiel Jewett, served as secretary of the fledgling University of California, and was appointed Assistant Curator of Mollusks at the United States National Museum (USNM) by S. F. Baird, where he worked until his death. Stearns published over 100 papers on "conchology," several dozen on various forestry and ethnology topics, and introduced species (Stearns, 1911). He named numerous molluscan species, including the tropical eastern Pacific *Conus dalli* in honor of his boss at the National Museum, William H. Dall. He named just two heterobranch species, the common white-on-white *Tritonia festiva* and the shelled *Acteon traskii,* the only extant sea slug originally named from fossil material.

William More Gabb (1839–1878) served as the Paleontologist for J. Whitney's California Geological Survey. He described a number of marine shells that had been collected by J. G. Cooper during that Survey, including the umbraculid *Tylodina fungina*. His description was based on "a single specimen, fresh, although without the animal," from Santa Barbara Island.

3. 1875–1900: L.S.R. Bergh

Worldwide opisthobranch research during this period was dominated by one man, the prolific Danish physician Ludwig Sophus Rudolph Bergh (1824–1909). Dr. Bergh

worked as a medical doctor for 50 years in several Copenhagen hospitals. He specialized in the diagnosis, prevention and treatment of sexually transmitted diseases. When not working in medicine, he examined 1000s of specimens and published over 90 articles and monographs of sea slugs from around the world. As an anatomist he emphasized the comparative morphology of the nervous and reproductive systems and the radula in classifying nudibranchs and their related taxa. He described more than 500 species of sea slugs from around the world, but only 18 valid species from the eastern Pacific. Of these, 13 had type localities in the eastern Pacific, based on specimens dredged by the U.S. Fish Commission steamer ALBATROSS (*Elysia diomedea*), or sent to him by William H. Dall (*Felimida dalli*). One recently "re-discovered" species, *Akiodoris lutescens*, was collected by Dall from the low intertidal at Nazan Bay, Atka Island in the Aleutians; the site lies just barely within the eastern Pacific, at 52.232° N; 174.1726° W. Bergh noted, "The fauna of the North Pacific in general has been but little explored. The number of the so-called Nudibranchiate Gasteropod [sic] Mollusca found in this region up to this time is rather small...and the number of forms is much smaller than that which is known from the North Atlantic in the same latitudes. There does not, however, seem to be any reason for a smaller number in the Pacific than in the Atlantic" (Bergh, 1879). Researchers from California and Canada in the early twentieth century proved his comments correct.

Upon Bergh's death, Dall (1909) wrote that he "was most genial and agreeable in manner, ever ready to help younger students or serve as cicerone to foreign colleagues.... [A] staunch friend."

4. 1901–1925: Canada and California

This period is distinguished from previous years by a group of researchers who spent significant periods of time living and collecting on the west coast, from California to Canada. By far the most emphatic proponent of opisthobranch research was Frank Mace MacFarland (1869–1951). He was born in Centralia, Illinois, and after receiving his bachelor's degree he was hired as a professor of biology and geology at Olivet College in Michigan. A few years later, the ichthyologist David Starr Jordan hired him as an instructor in histology at Stanford University. While teaching, he earned his master's degree there, and then a Ph.D. in Germany from the University of Würzburg. Returning to Stanford, he advanced to Professor of Histology. Meanwhile, he was instrumental in the founding of Hopkins Marine Station in Pacific Grove, serving as co-director from 1915–1917. MacFarland retired from Stanford in 1934 and was elected President of California Academy of Sciences, a post he filled until 1946. He had met his wife, Olive Knowles Hornbrook (1872–1962) in Indiana on his way to Germany. She was an accomplished zoologist and a gifted artist, producing meticulous drawings that accompanied his publications. In the introduction to MacFarland's posthumous 1966 opus, then-President of California Academy of Sciences, Robert C. Miller wrote simply, "[He] was one of the kindest and friendliest of men. He radiated cheerfulness and goodwill."

During the first quarter of the 20th century, MacFarland described 18 species of nudibranchs, mainly from intertidal regions around Monterey Bay and Pacific Grove (Figure 11). His description in 1925 of the pungently aromatic *Acanthodoris lutea* was based on two specimens, one of which had been collected by Dr. Myrtle Elizabeth Johnson at Cayucos, in San Luis Obispo County. She and Harry James Snook (1927) published *Seashore Animals of the Pacific Coast*, the first comprehensive guide to the common seashore animals of the west coast of the U.S. Although their book included color drawings of 20 nudibranchs, neither they nor MacFarland ever published a color painting of this vivid, bright orange species.

The importance of MacFarland's contributions to our knowledge of eastern Pacific heterobranchs, along with his enthusiasm and inspiration to others, cannot be overemphasized.

The Englishman Charles H. O'Donoghue (1885–1961) was a professor at the University of Manitoba and Director of the Marine Biological Station at Nanaimo on Vancouver Island. He returned to the British Isles in 1928, teaching at Edinburgh and Reading University. In a series of papers between 1921–1927, he described the nudibranch fauna of the Vancouver Island region, naming nine new species. He reported on a collection of 28 species of nudibranchs collected by W.A. Hilton and students from Laguna Beach. Hilton was the director of the Laguna Marine Laboratory (Pomona College, Claremont) and encouraged nudibranch research by his students (see Bacon, 1913). He was honored with O'Donoghue's *Phidiana hiltoni*.

Theodore Dru Cockerell (1866–1948) was also born in England but had to leave for health reasons. He spent most of his professional life at various museums and universities in Jamaica, New Mexico, and Colorado, and became a U.S. citizen. Primarily an entomologist, he was a prolific namer of living and fossil insects, mollusks and plants, and other taxa. He named over 6,000 species and genera of Hymenoptera, including a remarkable 34 million year-old wasp found embedded in amber from Colorado's late Eocene Florissant Formation. He and his second wife, Wilmatte Porter Cockerell, traveled widely, even crossing Russia on the Trans-Siberian Railroad. They were in Japan during the Great Kantu Earthquake of 1923. This 7.9 magnitude quake devastated Tokyo and Yokohama and generated a tsunami of 10 m which struck the coast of Sagami Bay. Years later in this bay, Emperor Hirohito collected the nudibranchs described, illustrated, and named by Kikutaro Baba (1949, 1955). Cockerell's visits with his wife to tide pools in southern California resulted in the collection of a number of new species. He and MacFarland reciprocated patronyms. Cockerell also named species in honor of his wife, J. G. Cooper, and R. E. C. Stearns.

The peripatetic British ambassador, colonial commissioner, and University Vice-Chancellor, Charles Norton Edgecumbe Eliot (1862–1931), published numerous

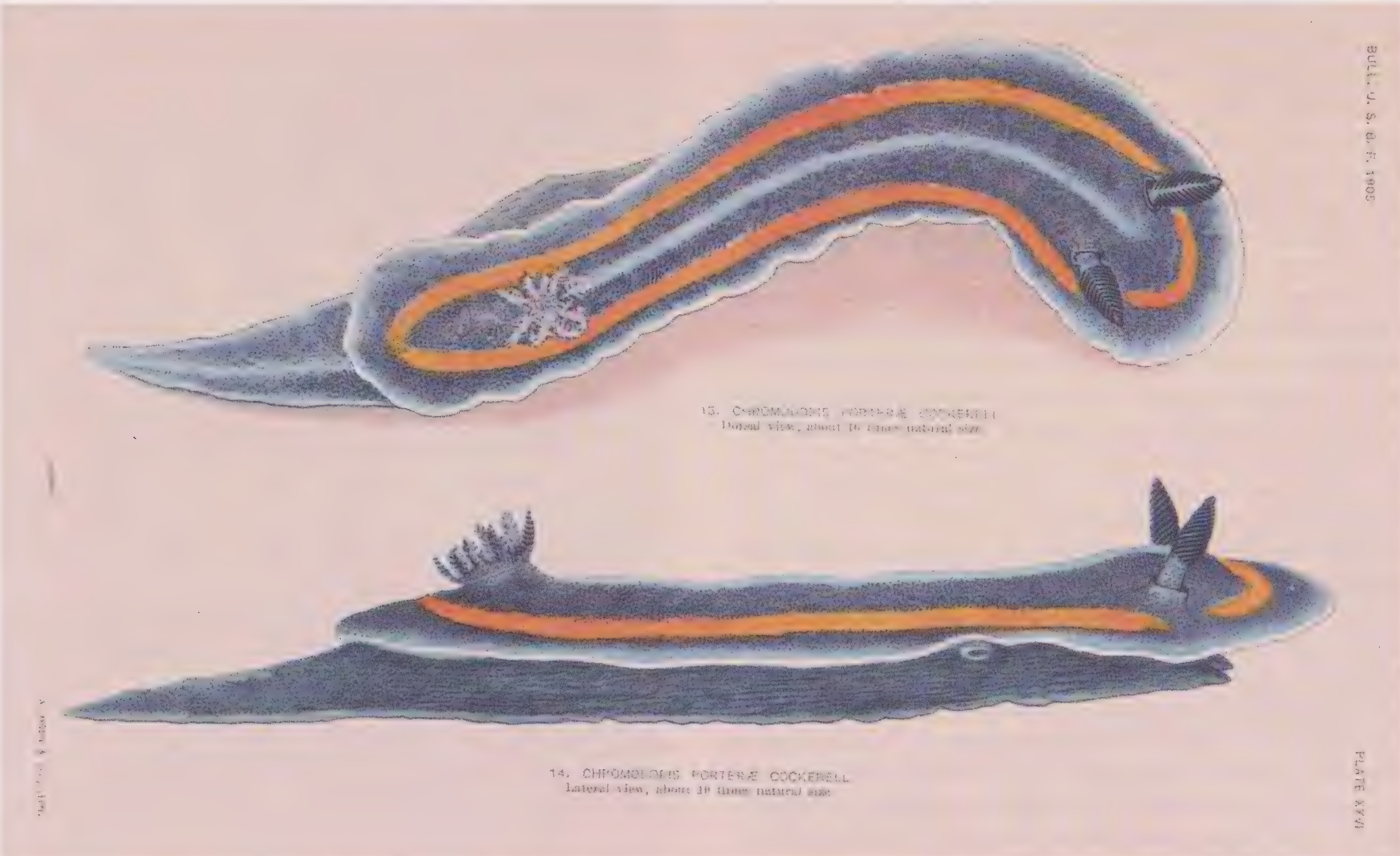

Figure 11. An original painting of *Chromodoris porterae* Cockerell, 1901, by Anna B. Nash, artist of the Hopkins Seaside Laboratory. (MacFarland, 1905: Plt. XXVI)

descriptions of nudibranchs, especially from Antarctica and the Indian Ocean. Cockerell and Eliot (1905) reported on a collection of Californian nudibranchs, resulting in five new species and a genus with authorship cited as "Cockerell in..." (e.g., *Anteaeolidiella chromosoma*) or "MacFarland in...." (e.g., *Dirona picta*).

William Healey Dall (1845-1927) is a bit difficult to place within one period, since his publications spanned decades, from 1866 to (posthumously) 1938, and he interacted with numerous researchers. He is considered here because he named most of his heterobranch species (14 of 18) during this time period. Like so many other workers up to this period, his primary focus was not specifically on sea slugs. For instance, he not only named a species of abalone (*Haliotis*), but had one named in his honor. A decades-long curator of mollusks at the USNM, Dall was a profligate namer of genera and species, totaling (including the synonyms) 5,302 molluscs and 125 non-molluscan invertebrates (Boss, Rosewater, and Ruhoff, 1968). This was a tremendous amount of names to devise, but only 27 names were used more than ten times. His most common patronym (used for 17 species) was *stearnsii*, honoring his contemporaneous fellow mollusk curator at the USNM, Robert Stearns. Dall also named the genus and species of the barnacle *Cryptolepas rachianecti*, which lives only on the California gray whale, annually migrating from Alaska to the Pacific lagoons of Baja California Sur. Dall had made a number of expeditions to Alaska, both before and after the U.S. had purchased it from Russia.

Based on two pale green empty shell valves collected by Charles R. Orcutt from Bahía Magdalena, Dall (1918) named the "clam" *Scintilla chloris*. Since the late 19th century, fossil shells of Juliidae had been considered mytilid clams. However, Kawaguti and Baba (1959) found living specimens of this group in Japan, among the green *Caulerpa* alga. They described the animals correctly as bivalved sacoglossans. Dall's species is now *Berthelinia chloris*, properly ensconced as a sacoglossan heterobranch. Some years earlier Dall (1884) had named the southern California "naked" clam *Chlamydoconcha orcutti*. The collector and describer had unwittingly collaborated in a polar coincidence: a bivalve slug and a slug-like bivalve! Orcutt (1864–1929) was a San Diego cactologist and malacologist, instrumental in founding the San Diego Natural History Museum. He traveled extensively throughout northern Baja California and elsewhere (DuShane, 1971), eventually dying in Haiti from malaria.

5. 1926–1960: An Inter-Period

Eastern Pacific opisthobranch research endured a slowdown of activity during this period. Although Japanese (Kikutaro Baba), French (Alice Pruvot-Fol) and Swedish (Nils Odhner) authors were publishing major papers on

new species of opisthobranchs globally, only 11 species were named from material that was originally found in the eastern Pacific. MacFarland and/or O'Donoghue named five of these, based on their earlier collections. Two aplysiids, three shelled species, and one noteworthy nudibranch constitute the rest. The nudibranch was a patronym, named by his colleague G. Dallas Hanna: "A few minutes before Dr. F.M. MacFarland collapsed on February 21, 1951, he discussed with me the generic position of a rather remarkable species of nudibranch.... He unquestionably would have described this animal in his very thorough manner had fate permitted. As a poor substitute, I will endeavor to place it on record and it seems fitting that it be named for him," *Platydoris macfarlandi* Hanna, 1951.

Several other regional publications on the natural history and biodiversity of sea slugs deserve mention. Costello (1938) described and illustrated the egg masses and reproductive periodicity of 22 species of nudibranchs in the Monterey Bay region, and Steinberg (1954) published a dichotomous key identifying 50 of California's more common opisthobranchs. *Sea of Cortez: A Leisurely Journal of Travel and Research* (Steinbeck and Ricketts, 1941) did not immediately garner the attention it deserved since it was published on the 6th of December, the day before Pearl Harbor was bombed by the Japanese, which plunged the United States into the global war effort. Today, this book is acknowledged as the pioneering research publication concerning the living marine invertebrates of the Gulf of California. This collaboration between a Nobelist in Literature, John Steinbeck (1902–1968), and a Monterey Bay marine biologist, Edward F. Ricketts (1897–1948) describes their six-week expedition doing science, and the people they met and the places they visited, as well as including a scientific catalogue of the specimens they observed or collected. In this work they discussed the natural history and biogeography of 14 species of heterobranchs, including a "large seal-brown nudibranch" (possibly *Cadlina luarna*). They correlated regional and seasonal temperature variations of the waters of the Gulf with the co-occurrence of fauna from the southern tropical regions of the Panamic province and the northerly temperate California province, being the first investigators to comment on the ecotonal nature of the Cortezian province.

6. 1961–2000: Coming of Age

Possibly the surge in opisthobranch research in 1961 and the founding of the molluscan journal *The Veliger* by Dr. Rudolf Stohler was a synergistic coincidence. Numerous articles on eastern Pacific heterobranchs have been published within its pages. Between 1959–1961 resident researchers Joan E. Steinberg and James R. Lance published a series of papers in *The Veliger* updating the nomenclature and known distribution of opisthobranchs from the west coast of North America. Steinberg is now retired, having spent her career as an award–winning elementary and middle school teacher in San Francisco. Lance (1928–2006) lived for decades on Agate Street in La Jolla, just blocks from his long-term intertidal collecting area. He worked as a laboratory technician in the laboratory of Dr. William Fenical at Scripps Institution of Oceanography (SIO), tending algae and collecting invertebrates for chemical studies in marine pharmacology. Five species of nudibranchs and sacoglossans that he described from the eastern Pacific are still considered valid.

In a 1961 supplement to volume 3 of *The Veliger*, Ernst Marcus described the anatomy and distribution of 50 species of opisthobranchs from California, from which he named 11 new species (five are still considered valid). The specimens had been collected by the Brazilian invertebrate zoologist Diva Diniz Corrêa (1918–1993) while she was on a Guggenheim Fellowship at the Pacific Marine Station in Dillon Beach and SIO. Joel W. Hedgpeth (honored with *Elysia hedgpethi* Marcus, 1961) helped her collect. She had received her doctorate in 1948, under the direction of Ernst Marcus. Upon his retirement, she was appointed to his Chair, eventually serving as the first female director of the Department of Zoology at the Universidade de São Paulo.

Ernst and Eveline Marcus had both been born in Berlin (in 1893 and 1901, respectively). He actually served in World War I as a German soldier. After receiving his doctorate in 1919, he was a museum curator of bryozoans and then university professor. He married Eveline Du Bois-Reymond in 1924, and they began their remarkable scientific collaborations spanning decades and continents. Anti-Jewish sentiment dismissed him from his professorship, and in 1936 they fled Nazi Germany to Brazil, where he was appointed a professor of zoology at the Universidade de São Paulo. Their earliest investigations in their newly-adopted country were on "lesser known" invertebrates such as bryozoans, nemerteans, and tardigrades. He was honored with Brazilian citizenship in 1940. However, during the height of World War II, because of his German ancestry, he was not allowed to study nor visit the coast, so he concentrated on freshwater invertebrates. Ernst and Eveline began studying opisthobranchs in the early 1950s. After his death in 1968, Eveline Marcus continued publishing, producing some 30 works on her beloved opisthobranchs. She even visited the San Diego area, meeting and collecting with various local researchers, before her death in 1990.

Pioneering work by Wesley M. Farmer and Clinton L. Collier in the early 1960s investigated the waters of northwestern Mexico. They named four still-valid species from the Gulf of California and Islas Cedros (Farmer, 1963; Collier and Farmer, 1964) and reported on the distributions of opisthobranchs along the Pacific coast of Baja California (Farmer and Collier, 1963). These were the first nudibranch species named from specimens collected in Mexico's Sea of Cortez. Note that Lance's (1962) *Histiomena convolvula* has been synonymized with *Histiomena marginata* Ørsted in Mörch, 1859.

Shortly afterward, Marcus and Marcus (1967) published the first comprehensive report of opisthobranchs

from the Gulf of California, noting that its nudibranch fauna was not well known. They examined a collection of animals sent by Peter E. Pickens, from the University of Arizona's field station at Puerto Peñasco, Sonora. Seventeen of the 20 new species they proposed are still recognized today.

Returning to studies of heterobranchs in central California waters, MacFarland's (1966) posthumously published monograph introduced over a dozen new species. Along with the Marcuses (1961 and 1967) publications, it stands among the three pivotal works that "jump-started" modern nudibranch research in the eastern Pacific. In 1969 and 1970, Richard A. Roller published several taxonomic updates to MacFarland's monograph. Roller (1930–1998) had taught high school biology in San Luis Obispo for years before moving back east to indulge his interest in antique fruit and canning jars. Early in 1969, he and Steven J. Long co-founded the *Opisthobranch Newsletter*, a monthly mailing of news, notes, references, and queries and comments by various investigators. In various iterations, this useful pre-Facebook communication traveled to the worldwide opisthobranch researchers under the face of a postage stamp.

Checklists of records of nudibranch species from Santa Barbara (Sphon and Lance, 1968) and San Luis Obispo Counties (Roller and Long, 1969) gave a better understanding of the distributional patterns for nudibranchs along the California coast. Gale G. Sphon (1934–1995) worked as a curatorial assistant in the Santa Barbara Museum of Natural History and the Los Angeles County Natural History Museum. (On a very personal note, Gale was responsible for introducing this author to nudibranchs and their intertidal brilliance.) Working alongside these Californian investigators, Gary McDonald, Dave Behrens, Terry Gosliner, and others began what would be lifelong (and still ongoing) studies on the taxonomy and natural history of Heterobranchia.

While visiting Friday Harbor Laboratory, the English investigator Anne Hurst (1967) described the egg masses and veliger larvae of 30 species of opisthobranchs from the San Juan Islands, Washington. She illustrated and defined the patterns of their developmental shapes.

Research in the Gulf of California yielded additional new species from that region (e.g., Farmer, 1978). With a type locality in the southern Gulf, *Chromodoris baumannni* (now in the genus *Felimida*) was the first species of nudibranch ever named using scanning electron microscopy to illustrate the radula (Bertsch, 1970). The first 3-D images of the teeth were published as stereo SEMs in the description of another Gulf species (Bertsch et al., 1973). Line drawings from light microscopes were replaced with this new technology, which has become the standard for illustrating the teeth and jaws of heterobranchs.

In situ field studies started to give a better understanding of nudibranch ecology. Gordon Robilliard (1971) used scuba diving to document subtidal ecology in Puget Sound. James W. Nybakken (1936–2009), professor of biological sciences at the Moss Landing Marine Laboratory, studied the intertidal community at Asilomar State Beach (Monterey Peninsula). His studies (Nybakken, 1974, 1978) were the first quantitative studies of nudibranch assemblages on the Pacific coast. Over a 40-month period, he used a timed-search methodology to calculate seasonal and yearly variations of slug densities. These results have been used to contrast findings at other eastern and central Pacific sites (see Bertsch, 2011). For most nudibranchs, using a timed search gives a better accounting of their occurrence and density than does the traditional transect/quadrant method. At Cape Arago, Oregon, Jeffrey H. R. Goddard (1984) examined the ecology and natural history of 46 species of opisthobranchs, including descriptions of the egg masses and larval development for 21 of those species.

Numerous experimental works were published on the neurophysiology and behavior of *Hermissenda* (e.g., Alkon, 1980), *Tritonia* (e.g., Lohmann et al., 1991), and *Aplysia*. Eric R. Kandel won the 2000 Nobel Prize in Physiology of Medicine for his research on "signal transduction in the nervous system" (Kandel, 2006). He proposed the basis for memory in complex systems such as the human brain by studying synaptic changes involved in learning and memory in *Aplysia californica*, a far simpler system. He isolated neuronal connections (before and after conditioning) in the ganglia of *A. californica*. When he was a youngster of 9 years old, he and his family had fled the anti-Semite Nazi persecution in Vienna, settling in New York. After training in medicine and a residency in psychiatry, he began his research into the mechanistic basis of learning. Multiple other species have been used in pharmacological studies, looking for anti-viral, anti-bacterial and anti-cancer chemicals (e.g., Turner et al., 1998).

This period also marked a new high in the naming of new species from the eastern Pacific, averaging almost three per year. Papers by David W. Behrens and Terrence M. Gosliner resulted in the description of some two dozen new species and several new aeolid genera such as the pretty *Hermosita*, and the Baja California-based *Bajaeolis* (Gosliner and Behrens, 1986). Among other new species were two highly appropriate patronyms: *Eubranchus steinbecki* and *Catriona* (now *Tenellia*) *rickettsi*. Continuing the international nature of eastern Pacific marine heterobranch research, Antonio Mozqueira Osuna was the first Mexican citizen to name a species of nudibranch, *Tritonia myrakeenae*. A student at Ciencias Marinas, Universidad Autónoma de Baja California, he had collected several paratypes from El Sauzal (several kilometers north of Ensenada), while researching his Tesis de Licenciatura.

7. 2001–Present: DNA, and Taxonomic and Spatial-Temporal Changes

A. Understanding taxonomy from a pan-oceanic perspective

The Swedish botanist C. Linnaeus classified living beings in a hierarchical scheme and introduced binomial

nomenclature, simplifying the human desire to name. Dating from his 1758 edition of *Systema Naturae*, this system is still in use. In the next century, the basis for classification changed from just shared morphological similarities to evolutionary relationships. Lamarck's 1809 and Darwin's 1868 drawings are the first know phylogenetic trees (Wheelis, 2007). Rudolph Bergh (1890) published the first phylogeny of nudibranchs (Figure 12).

As global nudibranch research intensified during the 20th century, numerous classifications were proposed by

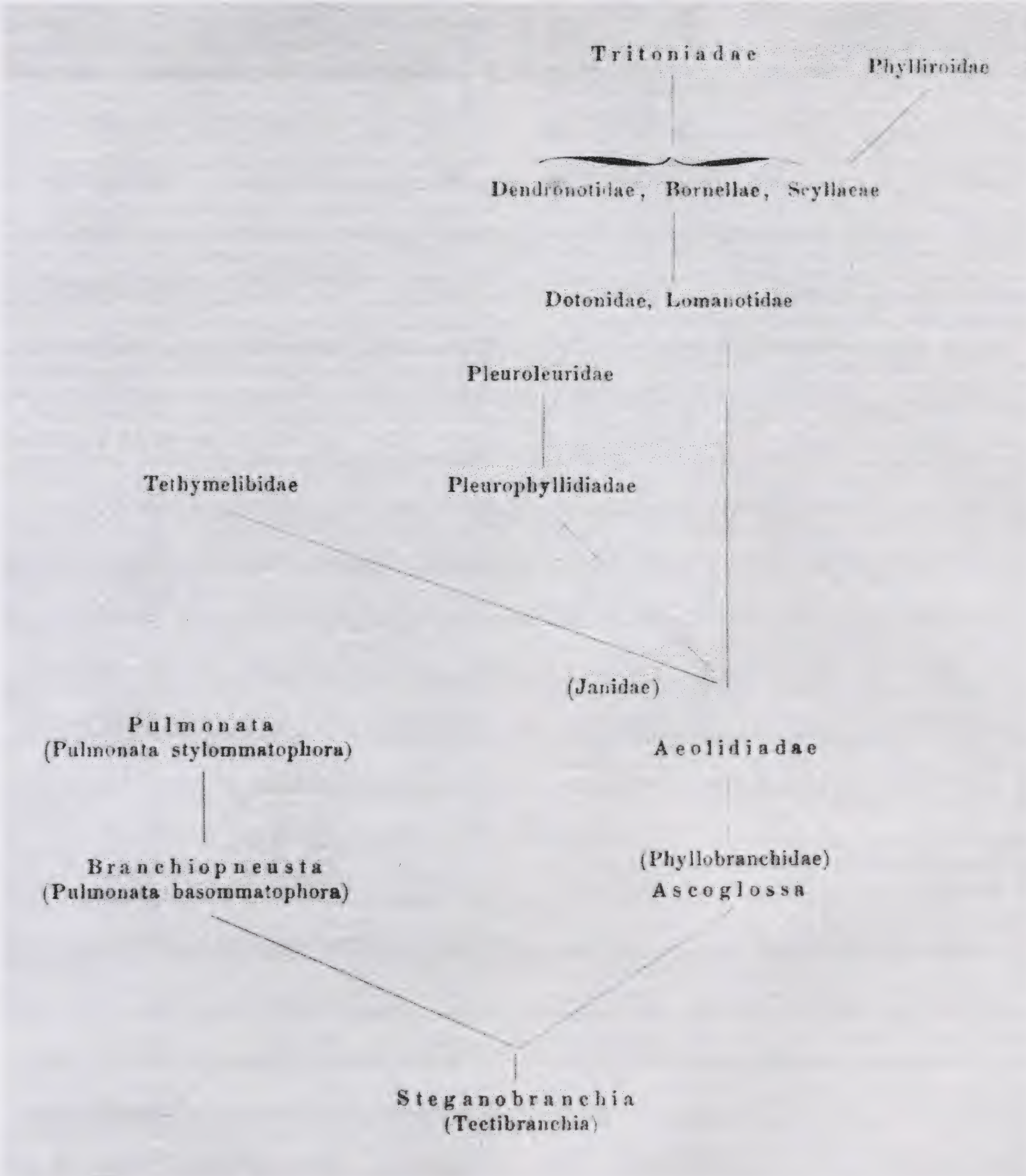

Figure 12. First phylogenetic tree of nudibranchs. (Bergh, 1890: 7)

European researchers to explain the evolutionary relationships within this group, e.g., the Belgian Pelseneer (1894), the Swedish Odhner (1934), and the German Boettger (1955). In a comprehensive study of opisthobranch phylogeny, Ghiselin (1966) used a functional analysis of the reproductive system "to take causes of evolutionary change into account in reconstructing the sequences of modification."

In the 1980s, phylogenetic studies of Opisthobranchia were revolutionized by the use of Hennigian calculable phylogenies. Hennig (1966) had proposed analyzing shared and derived characters to determine the evolutionary relationships among taxa at various hierarchical levels. Cladistic analyses based on morpho-anatomical characters were used to describe relations between major subgroups, families and genera (see Wägele et al., 2014, for an exhaustive list). Especially noteworthy was Gerhard Haszprunar (1985) formally establishing Heterobranchia as a phylogenetic clade. A major taxonomic upheaval occurred as phylogenetic studies gained a new tool, the genetic analysis of RNA and DNA sequences.

The first genetic analyses of phylogeny (e.g., Tillier et al., 1994; Thollesson, 1999a, b) used only a few mitochondrial RNA genes, based on a small sampling of species. Soon additional genes were studied, from a greater breadth of species. The monophyly of Euthyneura and Opisthobranchia were debated (e.g., Grande et al., 2004a, b; Vonnemann et al., 2005; Dinapoli and Klussmann-Kolb, 2009) and different groups were included or excluded from traditional clades.

Multiple studies showed the use of the clade Opisthobranchia in the traditional sense was no longer acceptable (Thollesson, 1999b; Schrödl et al., 2011). Sacoglossa was separated from the traditional orders of Opisthobranchia and placed as an early divergence within the Pan-pulmonate lineage. Nudibranchs are no longer the "crown" of the Cephalaspidea-Anaspidea-Notaspidean lineage. Gone is the tri-partite division of Gastropoda into Prosobranchia, Opisthobranchia, and Pulmonata; gone are the Opisthobranchia with their five major orders. Who's related to whom is no longer what had been thought (Wägele et al., 2014; Kano et al., 2016).

B. Eastern Pacific Studies and International Cooperation

Eastern Pacific sea slugs were quickly caught up in this new gene-based taxonomic volatility. Four California museum or university laboratories were dedicated to heterobranch research: MacFarland's modern California Academy of Sciences (under Terrence M. Gosliner), Natural History Museum of Los Angeles County (Ángel Valdés, currently Jann Vendetti), California State University at Los Angeles (Patrick J. Krug), and California State Polytechnic University at Pomona (Á. Valdés). Independently or collaboratively, researchers from Europe also studied eastern Pacific nudibranchs and their allies, including Alexander Martynov and Tatiana Korshunova (Moscow State University, Russia) and J. Lucas Cervera (Universidad de Cádiz, Spain). These investigators and their students have studied families, genera, and species occurring in multiple circumglobal marine provinces, including those in the eastern Pacific. Over 60 species of Heterobranchia have been named from the eastern Pacific during this historical period. Species thought to have occurred in multiple provinces have been named as new, with restricted distributional patterns of the different populations. Eastern Pacific specimens originally identified as the Mediterranean *Limenandra nodosa* Haefelfinger and Stamm, 1958, are now *Limenandra confusa*, and the Caribbean *Polybranchia viridis* (Deshayes, 1857) from our coastline is now *Polybranchia mexicana*. Even along the North American Pacific coastline, cryptic and pseudo-cryptic species have been discovered with differing northern and southern distributional patterns. For some (e.g., *Diaulula odonoghuei* and *Hermissenda opalescens*) prior synonyms have been restored; for others, new species names have been proposed (e.g., *Limacia mcdonaldi* and *Doriopsilla davebehrensi*). There have been scores of taxonomic changes since the publication of *Eastern Pacific Nudibranchs* (Behrens and Hermosillo, 2005), which have necessitated a new, fully revised and updated edition. Admittedly, individually mentioning each and every one of these contributions would be excessive. Hence, only a few representative papers and authors have been highlighted here.

Research in the subtropical and tropical waters of the eastern Pacific now includes the field work of Mexican and Costa Rican workers studying their nations' fauna. After completing graduate student theses, Alicia Hermosillo McGowan (studying the heterobranchs of Bahía de Banderas, Jalisco-Nayarit, Mexico), Orso Angulo Campillo (at La Paz, Baja California Sur, Mexico), and Yolanda Camacho-García (Costa Rica) have gone on to name several dozen nudibranch species from these waters (e.g., *Polycera kaiserae*, *Marionia kinoi*, and *Jorunna osae*). The active work of these and other Latin American investigators have greatly enhanced international research efforts across marine faunal provinces and nations in the eastern Pacific.

Long-term subtidal natural history studies using Nybakken's (1978) timed-search method (Hermosillo, 2006, three years; Bertsch, 2019, 30+ years), have yielded information on annual and seasonal variations, feeding, reproduction, and bathymetry of heterobranch species communities in the Gulf of California and along the southwestern Mexican coastline.

Using data from Jim Lance's La Jolla surveys and more recent observations throughout southern California, the extinction and recovery of the brilliant blue and gold nudibranch *Felimare californiensis* has been documented (Goddard et al., 2013; Hoover et al., 2017).

For years, species common in the Gulf of California have been sporadically reported from southern California. Species such as *Pleurobranchus digueti*, *Berthellina ilisima*, and *Flabellina bertschi* have been cited as "El Niño occurrences," but without confirmatory timed data sets available. Recent work by a cadre of researchers in the Californian and Oregonian faunal provinces has correlated

northward range shifts of heterobranch species with distinct periods of El Niño phenomena (Goddard et al., 2016, and 2018). With the anthropogenic-caused climatic changes endangering both terrestrial and marine habitats, these studies are important to determine adaptability and survivability of these organisms with range changes under climatic stress, and to formulate positive actions that can be taken by humans to ensure marine biodiversity conservation (Tittensor et al., 2019).

Our knowledge of eastern Pacific heterobranchs is growing and still being written. Conserving these organisms and their habitats is an essential part of that effort.

III.

"If facts are the seeds that later produce knowledge and wisdom, then the emotions and the impressions of the senses are the fertile soil in which the seeds must grow. The years of early childhood are the time to prepare the soil. Once the emotions have been aroused—a sense of the beautiful, the excitement of the new and the unknown, a feeling of sympathy, pity, admiration or love—then we wish for knowledge about the subject of our emotional response. Once found, it has lasting meaning."

Rachel Carson, 1965
The Sense of Wonder

The study of biology is historical (Mayr, 2004). It is a record of the evolutionary changes wrought by natural selection on the genotypic and phenotypic variations of living beings. The unique global ecosystem is a nexus of biological, chemical, and geological interactions, forming that synergy called life. Steinbeck and Ricketts (1941) argued against teleology when they wrote there is no why, that life just is. But because it is, it deserves to be.

Observation, hypothesis, experimentation and theory become science only with communication. The ethics of science demands truth in our research and in our communication. Predictability of the results ranges from the effects of mass and distance on gravity to the future of life in the Anthropocene. Predicting history can only be based on history. What will happen if human-caused global climate change, that has resulted in unwanted adverse changes and extinctions, continues? As in evolution, what is done today will create the future. Care for tomorrow is scientific. Conservation of earth's ecosystems and their biodiversity is science communicated well. Doing science is truly *for the children* and the future generations of all life. Whatever that future becomes depends on our research and actions today. The history will be written by the children.

ACKNOWLEDGMENTS

I am grateful to Robert Dees (Cambria, California) and Juan Lucas Cervera (Cádiz, Spain) for their generous and invaluable assistance with the writing of this manuscript, and to Jan Kocian (Whidbey Island, Washington) for allowing me to use his marvelous drawing.

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Research Note

First *ex-situ* observation of *Vasula deltoidea* (Lamarck, 1822) (Gastropoda: Muricidae) mating and egg-laying with emphasis on the potential for hatchery-reared individuals to aid coral reef restoration

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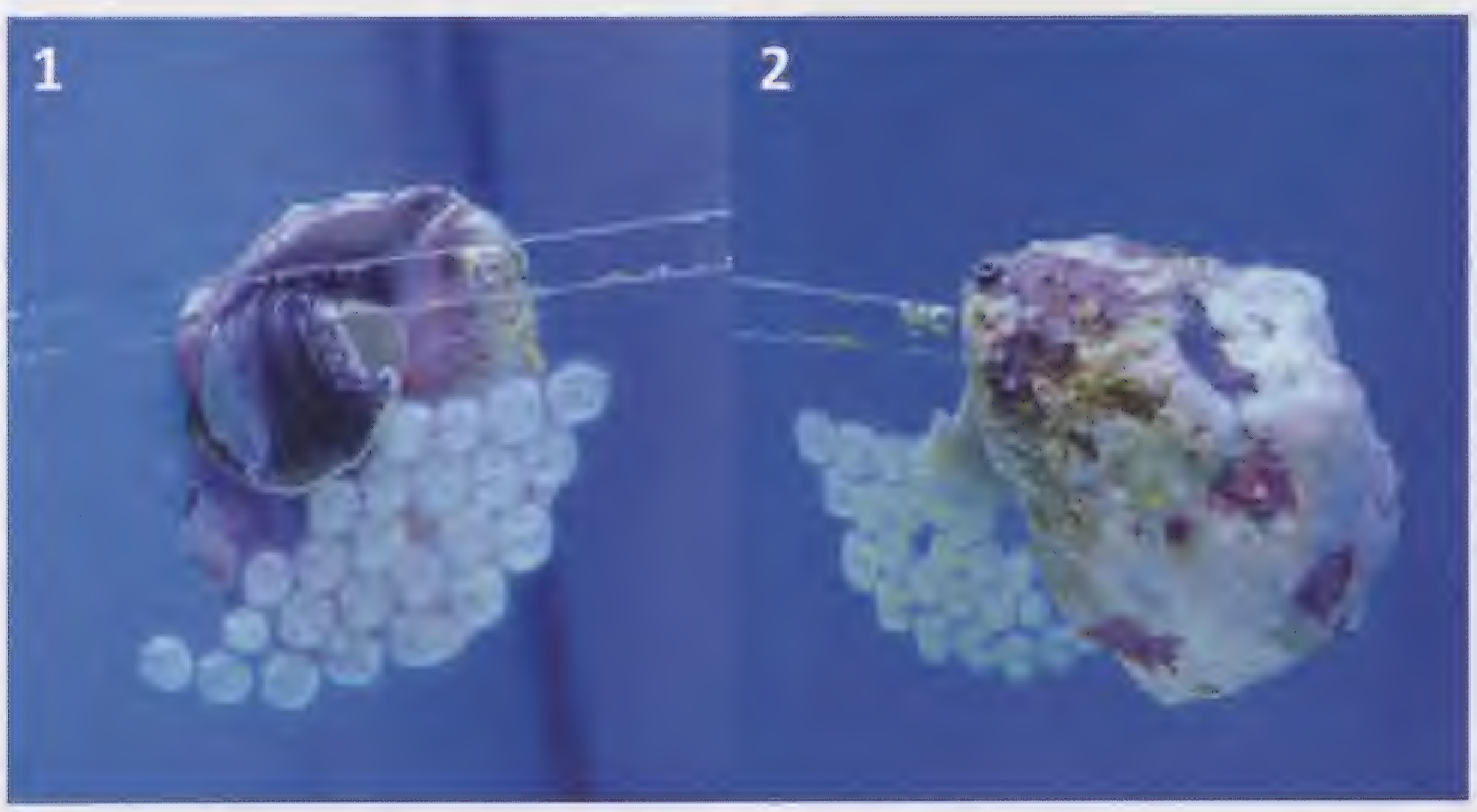
There is a long history of raising gastropods under hatchery conditions for stock enhancement and food production, but another application of gastropod aquaculture could be to aid coral reef ecosystem management and restoration (i.e., conservation aquaculture). Florida's coral reefs currently exist in a degraded state (Jackson et al., 2014), which has given rise to restoration efforts predominantly using outplanted staghorn coral, *Acropora cervicornis* (Lamarck, 1816) (Young et al., 2012; National Oceanic and Atmospheric Administration, 2020). However, the corallivorous gastropod *Coralliophila galea* (Dillwyn, 1823) is a major predator on acroporids (Potkamp et al., 2017a, b). Corallivory is a natural predator-prey interaction, but, in degraded coral reef ecosystems, corallivores can be a substantial impediment to recovery by creating synergies with other stressors thus augmenting coral mortality (Rice et al., 2019). To counteract this, the use of native, predatory gastropods to control corallivorous species has been espoused. For example, it has been suggested to have a ready supply of hatchery-reared Giant Triton snails, *Charonia tritonis* (Linnaeus, 1758), to deal with sudden outbreaks of Crown-of-thorns Starfish (*Acanthaster planci* species complex) on the Great Barrier Reef (Hall et al., 2017). In Florida, laboratory and field studies have demonstrated that the presence of the Deltoid Rock Snail, *Vasula deltoidea* (Lamarck, 1822), reduced the effects of predation by *C. galea* on *A. cervicornis* through direct consumption and also by provoking an escape response in *C. galea* (Delgado and Sharp, 2020).

To further these conservation aquaculture efforts, we describe herein the first *ex-situ* observation of *V. deltoidea* mating, egg laying, and early veliger growth. *Vasula deltoidea* individuals were collected from the Florida Keys reef tract and held for an acclimation period of at least one week under ambient nearshore seawater conditions. A temperature-controlled, 379-liter aquarium array (Marineland®) with centralized mechanical, biological, and chemical filtration was used for the trials. The array consisted of three separate 114-liter aquariums, each divided into three equal sections by perforated acrylic partitions, yielding a total of nine physically isolated sections all of which were subjected to the same recirculating water mass. Due to the difficulty in sexing individuals, each of the nine sections housed a pair of unsexed *V. deltoidea* that were monitored for up to 30 days. *Vasula deltoidea* were fed live *C. galea* and/or *Lithopoma americanum* (Gmelin, 1791). Trials were conducted at two temperatures, 23°C and 30°C, which typify winter and summer water temperatures along the Florida Keys reef tract, so that a total of 18 pairs were observed. The shell



Figures 1–2. *Vasula deltoidea* female in the process of laying the first of her egg clusters behind one of the slits in the acrylic partition in our aquarium array. The female and the egg cluster are both below the water line. **1.** Ventral view. **2.** Dorsal view.

lengths of these individuals ranged from 29.0–37.8 mm; the mean (± one SE) was 32.9 ± 0.31 mm. Synthetic seawater (Instant Ocean® Reef Crystals) at 35 ppt was used. The aquarium array had a flow rate of 4,540 liters per hour; lighting was on a 12-hour light-dark cycle and consisted of T5 10,000K and actinic fluorescent bulbs.

During the first ten days of one of the summer trials, we observed one pair of *V. deltoidea* copulating. The shell lengths of these two individuals were 32.7 and 34.8 mm. The female laid two clusters of egg capsules in four days (Figures 1–4). Each cluster took approximately 24 hours to complete. The first cluster contained 74 individual egg capsules, whereas the second had 41 (Figures 3–4). The capsules were round and flattened (Figures 3–4) and contained dark brown veligers (Figure 5). The number of developing veligers in five haphazardly chosen egg capsules was counted using a stereomicroscope. The mean number of veligers per capsule was 149.6 ± 4.28. Using this calculation and the number of capsules in each egg cluster, we estimated that the first cluster contained 11,070 ± 317 veligers; the second cluster had 6,134 ± 176. The water temperature in which the *V. deltoidea* and the developing veligers were held was 29.4 ± 0.05°C.

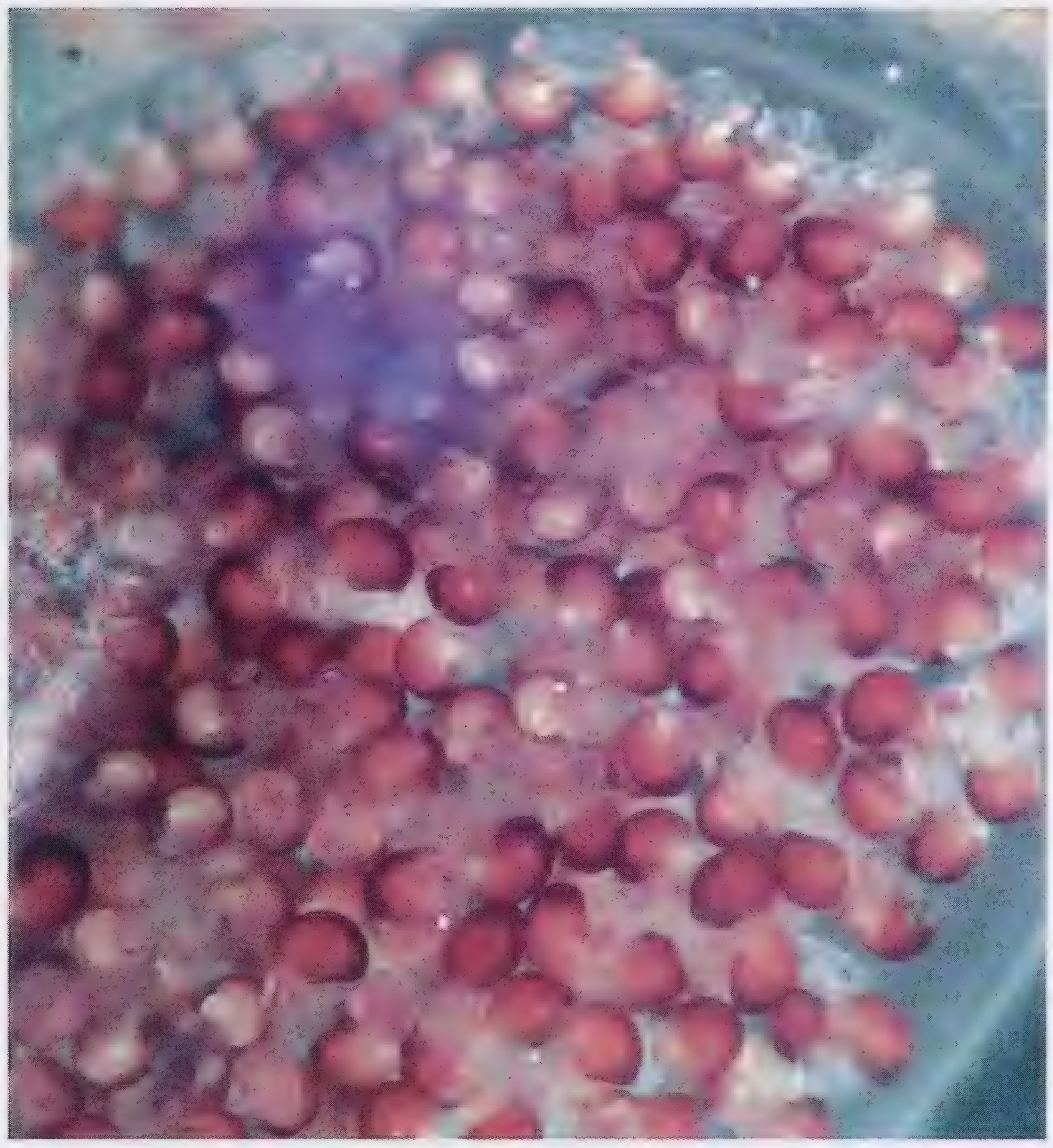
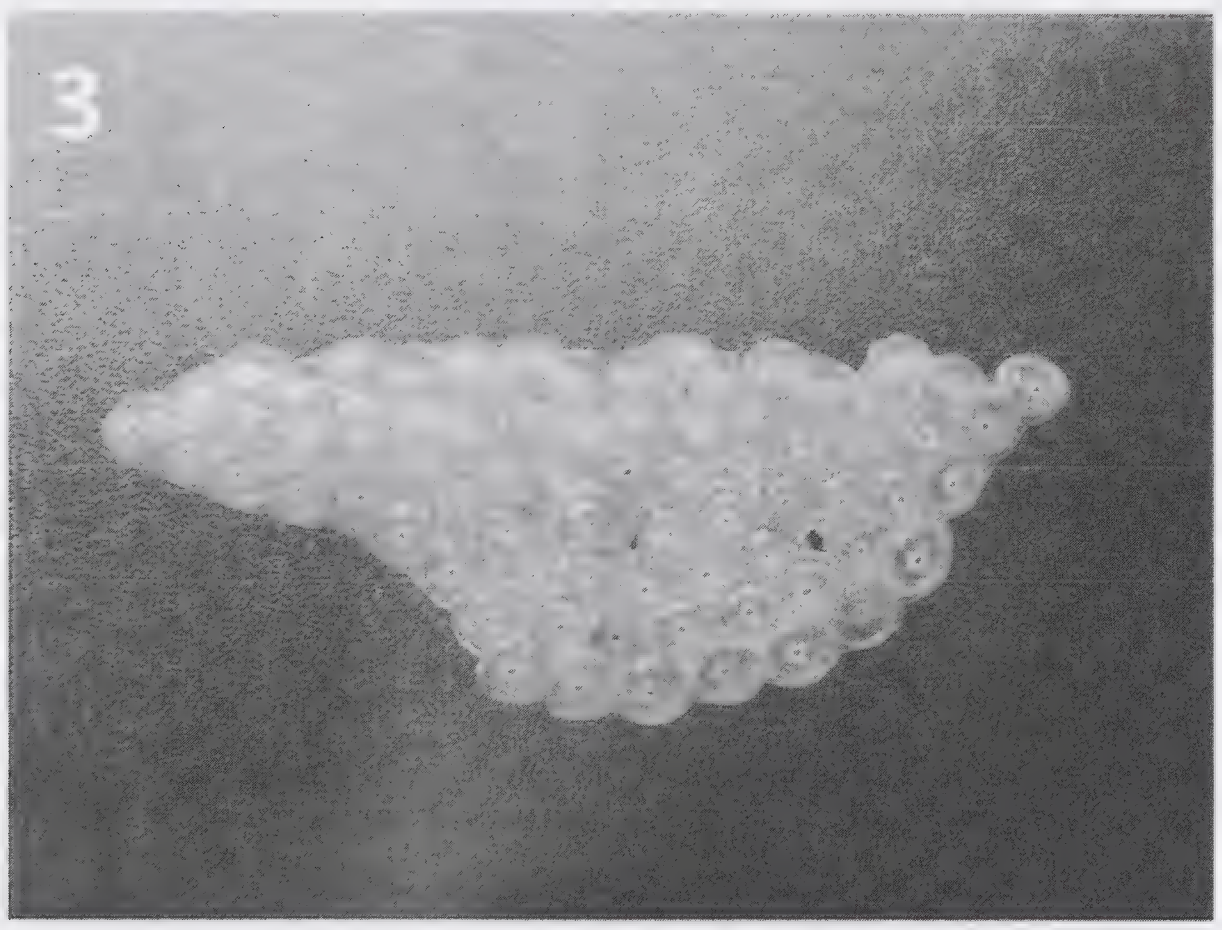


Figure 5. Photograph of the developing *Vasula deltoidea* veligers within one of the egg capsules; the veligers are 14 days old.



Figures 3–4. Post-hatch photographs of the two egg clusters laid by the *Vasula deltoidea* female. **3.** The first egg cluster contained 74 individual, adjacent egg capsules. **4.** The second egg cluster, laid 2 days later, had 41 egg capsules.

The eggs hatched after an incubation period of 21 days. The veligers from the first cluster were raised in a 21-liter aquarium. This equates to a stocking density of 530 veligers per liter. The veligers were fed approximately 4 ml of AlgaGen PhycoPure™ (a live mix of microalgal species) per day. To track growth, we measured the larval shell length of five haphazardly chosen veligers on days 1, 2, 3, 6, and 10. Newly hatched veligers were approximately 300 μm and grew slowly, reaching approximately 340 μm on

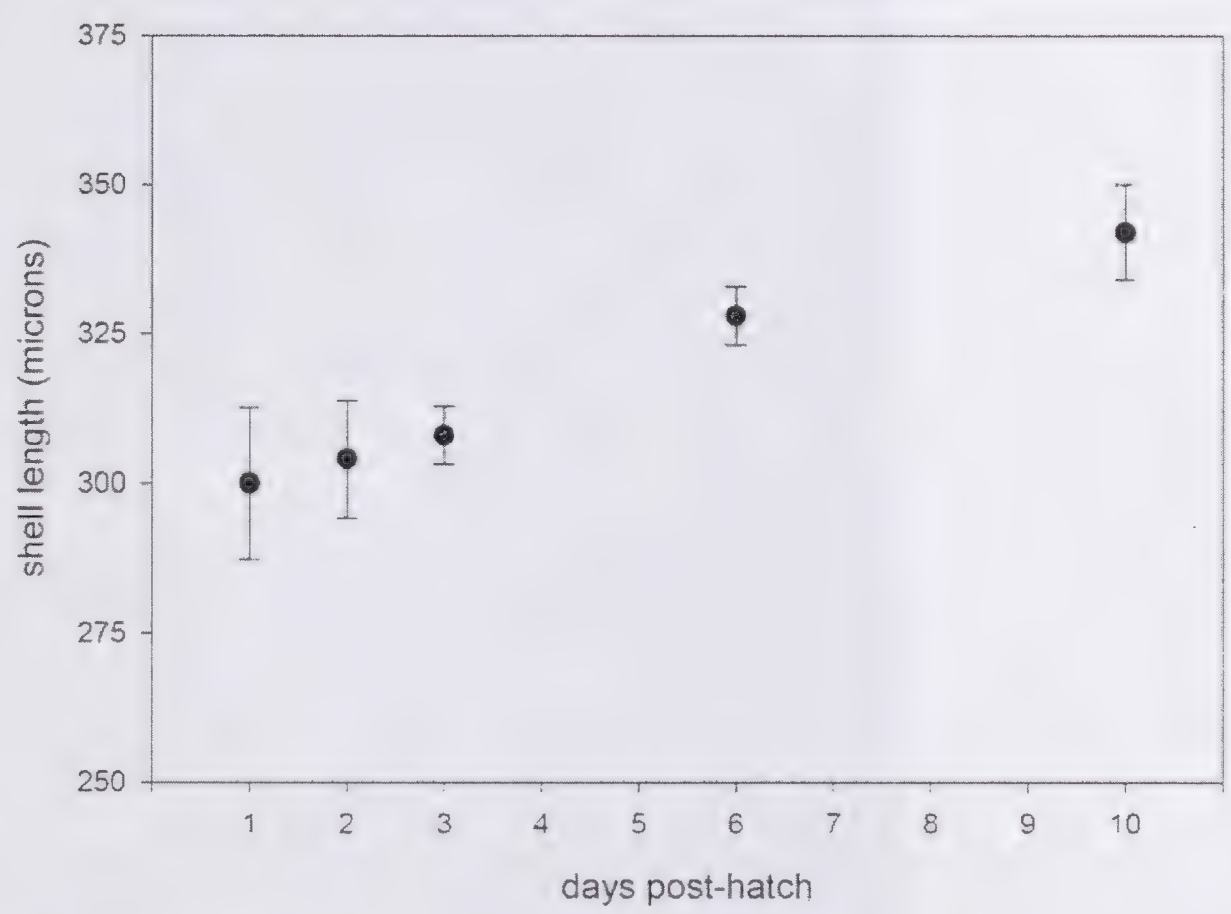


Figure 6. The mean shell length of the post-hatch *Vasula deltoidea* veligers over time. Error bars represent ± one standard error.

day 10 (Figure 6), after which all the veligers unexpectedly died. Water temperature was 29.8 ± 0.03°C; salinity was 35 ppt.

Our findings are consistent with the only other study (Lewis, 1960), as far as we know, that describes the reproductive aspects of *Vasula deltoidea* (Lewis, 1960). That study, conducted in Barbados, reported that *V. deltoidea* laid egg clusters when water temperatures were approximately 28°C, quite similar to our simulated summer temperatures. Lewis (1960) also observed that *V. deltoidea* laid egg clusters in holes and depressions below the water's surface. Our aquarium array consisted of smooth surfaces except for the perforations in the acrylic partitions, which provided *V. deltoidea* individuals a substitute for natural topography (Figures 1–2). We note that two other females laid egg clusters on the Vexar® mesh that covered the drainpipe in our acclimation tank, but no quantitative data were recorded in these instances. Evidently, the broodstock environment must have a measure of topographic complexity with holes or cavities in which females can lay their egg clusters. Nonetheless, further studies on broodstock maintenance are warranted. For example, broodstock conditioning can probably be improved through diet refinement as *V. deltoidea* will consume other mollusks besides *Coralliophila galea* and *Lithopoma americanum* (Delgado and Sharp, 2020). In addition, the frequency of copulation can probably be increased by sexing individuals or perhaps by keeping a group of more than two as *V. deltoidea* may prefer to mate *en masse* as in the confamilial *Thaisella chocolata* (Duclos, 1832) (Romero et al., 2004).

The physical characteristics that we detail for the *V. deltoidea* egg capsules and veligers are corroborated by Lewis (1960), but that study did not document development time or fecundity. The intracapsular development time of 21 days that we observed is comparable to other species within the subfamily (Romero et al., 2004). *Stramonita rustica* (Lamarck, 1822), a confamilial species also present in Florida, was found to have a similar number of veligers per spawn as our results; however, unlike *V. deltoidea*'s flat and round egg capsules, *S. rustica*'s egg capsules are vasiform in shape (D'Asaro, 1970). Lewis (1960) reported larger *V. deltoidea* veligers at hatch (360 μm) compared to those that we observed (300 μm), but the veligers in both studies survived only a few days post-hatch. Research into optimal water quality parameters, veliger stocking densities, and veliger nutritional requirements can probably improve survival. However, if *V. deltoidea* has a protracted (e.g., 3–4 months) larval phase like other members of its family (D'Asaro, 1970; Romero et al., 2004), raising the veligers to settlement may not be practicable even with improvements to larviculture techniques.

At present, most coral reef restoration efforts have been experimental or limited in spatial scale (National Academies of Sciences, Engineering, and Medicine, 2019). However, the Florida Keys National Marine Sanctuary is undertaking a massive project to restore seven reefs using almost 500,000 coral colonies, 40% of which will be acroporids (National Oceanic and Atmospheric Administration, 2020). Incorporating hatchery-reared *V. deltoidea* into reef restoration may increase the survival of the outplanted acroporids by mitigating *C. galea* corallivory (Delgado and Sharp, 2020). Our *ex-situ* observations of *V. deltoidea* mating and egg laying show some promise in this regard; however, further broodstock and larviculture studies are needed to determine if cultivating *V. deltoidea* for conservation purposes is feasible. If so, *V. deltoidea* may prove a valuable addition to coral reef ecosystem restoration efforts.

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Research Note

Range and dietary expansion of the nudibranch *Felimare ruthae* (Ev. Marcus and Hughes, 1972) (Gastropoda: Chromodorididae)

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Many nudibranch sea slugs have very specific diets; in particular, the family Chromodorididae Bergh, 1891 are oligophagous sponge feeders (Rudman and Bergquist, 2007). Many chromodorids only consume one species of sponge (Rudman and Bergquist, 2007) and rely on the sponge not only as food, but also for defensive secondary metabolites (Faulkner and Ghiselin, 1983; Cinimo and Ghiselin, 1999). The diet of many chromodorids, however, is poorly understood due to their rarity, poor documentation, and difficulty in identifying their sponge food (Rudman and Bergquist, 2007).

Recent studies, and the increase in popularity of nudibranch underwater photography and public records, have improved our knowledge of the geographic ranges of many species of nudibranchs. However, range boundaries are often not well established, and the distribution of certain species remains obscure. Recently, several nudibranch species, including chromodorids, have undergone range expansions (Özcan et al., 2010; Padula et al., 2011; Nimbs et al., 2016). Although some geographic shifts have occurred due to natural reasons (Borg et al., 2009), accidental introductions (Özcan et al., 2010; Lipej and Mavric, 2017) and climate change (Goddard et al., 2011; Nimbs et al., 2016) might have also contributed to the geographic spread of sea slugs.

Felimare ruthae (Ev. Marcus and Hughes, 1974) (Figure 1) is a small chromodorid ranging in size from 9 to 38 mm (Meyer, 1977; Humann, 1992). It is dark blue with 5 to 6 parallel lines that extend along the dorsum and a thick white marginal band with a bright yellow border (Ev. Marcus and Hughes, 1974; Ortea et al., 1996). The nudibranch is typically found at depths between 1–20 m in areas with moderate currents (Humann, 1992; Ortigosa and Simões, 2019). It occurs throughout the Caribbean, as far south as Venezuela and north as the Bahamas (Valdes et al., 2006). Additionally, it can also be found in the southern Gulf of Mexico, inhabiting coastal reefs of the Yucatan Peninsula (Ortigosa et al., 2013; 2015; Ortigosa and Simões, 2019). *Felimare ruthae* has been reported to feed on sponges *Dysidea janiae* Duchassaing and Michelotti, 1864 (Humann, 1992; Valdés et al., 2006) and *D. etheria* Laubenfels, 1936 (Rudman and Bergquist, 2007).

In June 2019, we were informed by local divers that *Felimare ruthae* had appeared in large numbers on limestone ledges located offshore of Clearwater, Florida, from where it had never been reported. These ledges are ~24 km offshore, rising 1–2 m off the seafloor in ~12–15 m depth, and supporting a diverse benthic community. On June 5, 2019, the area known as the Miss D Ledge (28°04.145′ N, 83°00.392′ W) was visited via SCUBA. *Felimare ruthae* was primarily found on medium to large demosponges. Up to five individuals were on a single sponge and several of them were observed feeding upon the sponges. On June 21, 2019, we conducted formal surveys of the sponges on that site. In total, 35 sponges were surveyed of which 12 harbored a total of 16 *F. ruthae*. Sponges were an average diameter of 56cm ± 13 cm. One slug was crawling on the limestone substrate. Tissue samples of several sponges were taken for identification. One other nudibranch, *F. picta* Philippi, 1836, also frequently occurs in this habitat feeding on species of *Dysidea* Johnston, 1842.

Morphological examination of the sponge tissue was conducted using the descriptions in Hooper and van Soest (2002). The sponge was lacking laminar structure, spicules, and fine collagenous fibers. The fibers present were homogenous suggesting it belongs in the family Spongiidae Gray, 1867. The sponge also had an unarmored surface, simple primary fibers without fascicles, and was not lacunose, suggesting it belongs in the genus *Spongia* Linnaeus, 1759.

DNA was extracted from an alcohol-preserved sample of sponge tissue using the DNeasy Blood and Tissue Kit (Qiagen). We used degenerate PCR primers to amplify the 28S rDNA C-Region (F: 5'-GAAAAGAACTTT-GRARAGAGAGT-3', R: 5'-TCCGTGTTTCAAGACGG-G-3') (Chombard et. al, 1998). A three-step PCR amplification program included initial denaturing at 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 45°C for seconds, and 72°C for 1 minute, and a final extension at 72°C for 5 minutes. Sanger-sequencing (Genewiz) was performed using purified PCR product. Sequence results were queried using the BLAST alignment tool. Results of the BLAST search showed that the lowest E-value of 5e-36 correlated with *Spongia* sp. (Genbank accession number KC869488.1).

Although chromodorids typically have very narrow diets, a few species have been documented consuming a wider variety of foods. For example, species in the genus *Ceratosoma* Adams and Reeve, 1850 exclusively eat dysideid sponges; however, *C. amoenum* Cheeseman, 1886 is also found feeding on species of the thorectid sponge *Semitaspongia* Cook and Bergquist, 2000 (Rudman

Figure 1. *Felimare ruthae* on *Spongia* species, at Miss D Ledge, off Clearwater, Florida.

and Bergquist, 2007). Species in the genus *Felimare* Ev. Marcus and Er. Marcus, 1967 seem to have evolved feeding on dysideid sponges (Johnson and Gosliner 2012); however, one Mediterranean species, *F. tricolor* Cantraine, 1835, sometimes consumes *Spongia* species (Furfaro et al., 2016). Either some chromodorid species can opportunistically feed on other sponges or specific populations have successfully changed diet.

Switching between diets, however, is not a trivial matter. The chemical metabolites produced by sponges can vary widely (Thoms and Schupp, 2007). For example, species in the family Dsyideidae produce sesquiterpenes, while those in the Spongiidae produce sesterterpenes (Rudman and Bergquist, 2007). By changing diets between these sponge families, *F. ruthae* not only has to adapt to metabolize a different suite of chemicals, but may also need to modify these metabolites for its own defense.

It is unclear what caused this range expansion, but the shift in diet may have contributed. It will be worthwhile to determine whether populations of *F. ruthae* from other areas also feed on *Spongia* species or if this is limited to Florida populations. It is unclear if this range expansion will be permanent; however, diver reports from early 2020 continue to indicate local occurrences of *F. ruthae*. Range expansions by other nudibranchs have been shown to modify community structure (Allmon et al., 1988), suggesting that continued monitoring would be worthwhile.

ACKNOWLEDGMENTS

We wish to thank Ryan Nelson, Seth Pikal, and Tanks-A-Lot Dive Charters for alerting us about the slug and assisting with surveys. We thank Tom Dix at Hillsborough County EPC for morphological identification of the sponge. The research was funded, in part, by a University of Tampa Summer Undergraduate Research Fellowship awarded to DGA.

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INSTRUCTIONS TO AUTHORS

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The first mention of a scientific name in the text should be accompanied by the taxonomic authority, including year. Metric, not English, units are to be used. The sequence of sections should be Title, Author(s) and Affiliations, Abstract, Additional Keywords, Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgments, Literature Cited, Tables, Figure Captions, Figures. If the author for correspondence is not the senior author, please indicate in a footnote. The abstract should summarize in 250 words or less the scope, main results, and conclusions of the article. Abstracts should be followed by a list of additional keywords. All references cited in the text must appear in the Literature Cited section and vice-versa. Please follow a recent issue of *The Nautilus* for bibliographic style, noting that journal titles must be unabbreviated. Information on plates and figures should be cited only if not included within the pagination of cited work. Tables must be numbered and each placed on a separate page. If in doubt, please follow a recent issue of the journal for sequence of sections and other style requirements.

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